Over-expression of denticleless E3 ubiquitin protein ligase homolog in human endometrial cancer.

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Gynecologic cancers including cancers of the endometrium are a clinical problem¹⁻⁴. We mined published microarray data^{5,6} to discover genes associated with endometrial cancers by comparing transcriptomes of the normal endometrium and endometrial tumors from humans. We identified denticleless E3 ubiquitin protein ligase homolog, encoded by DTL, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. DTL was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of DTL was correlated with overall survival in white patients with low mutational burden. DTL may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, DTL, denticleless E3 ubiquitin protein ligase homolog, systems biology of endometrial cancer, targeted therapeutics in endometrial cancer.

Endometrial cancer is the most common gynecologic cancer in the developed world¹. Over the last three decades, the incidence of endometrial cancer has increased 21%⁴ and the death rate has increased 100%³. We harnessed the power of independently published microarray datasets^{5,6} to determine in an unbiased fashion and at the systems-level genes most differentially expressed in endometrial tumors. We report here the differential and increased expression of the denticleless E3 ubiquitin protein ligase homolog (DTL) in human endometrial cancer.

Methods

We utilized datasets GSE63678⁵ and GSE17025⁶ for this global differential gene expression analysis of human endometrial cancer in conjunction with GEO2R. GSE63678 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=5$ control endometrial tissues (including $n=4$ uterine myomas and $n=1$ benign cyst) and $n=7$ endometrial cancers (including $n=2$ endometrial adenocarcinomas, $n=3$ mixed endometrioid adenocarcinomas, and $n=2$ adenocarcinomas with squamous differentiation); analysis was performed using platform GPL571. GSE17025 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=5$ normal endometrial tissues (inactive endometrium) and $n=91$ endometrial cancers; analysis was performed using platform GPL570. The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether DTL gene expression was significantly different between control endometrial tissue and endometrial tumor tissue in humans using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool⁷ for correlation of DTL mRNA expression levels with overall survival in $n=543$ endometrial cancer patients.

Results

We harnessed the power of blind comparative transcriptome analysis using published microarray data^{5,6} to discover in an unbiased fashion genes associated with endometrial cancer in humans.

DTL is differentially expressed in endometrial cancer.

We identified denticleless E3 ubiquitin protein ligase homolog, encoded by DTL, as among the genes most differentially expressed in cancers of the endometrium when compared to benign endometrial tissues (Chart 1). When sorting each of the genes expressed in endometrial tumor tissue based on significance of change in expression as compared to benign endometrial tissue, DTL ranked 184 out of 22273 transcripts, equating to 99.2% differential expression (Chart 1). Differential expression of DTL in human endometrial cancers was statistically significant (Chart 1; $p=2.29E-04$).

We queried a second microarray data to validate differential expression of DTL in endometrial cancer. Again, we observed differential expression of DTL when comparing endometrial tumor tissue to benign endometrial tissue (Chart 2). When sorting each of the genes expressed in endometrial tumor tissue based on significance of change in expression as compared to benign endometrial tissue, DTL ranked 274 out of 54675 transcripts, equating to 99.5% differential expression (Chart 2). Differential expression of DTL in human endometrial cancers was statistically significant (Chart 2; $p=2.42E-06$).

DTL is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for DTL in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of DTL in human endometrial cancer. DTL was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0005$). We calculated a mean fold change of 1.34 in DTL mRNA levels in human endometrial cancer, as DTL was expressed at 6.00 ± 0.60 arbitrary units (A.U.) in control endometrial tissue but at 8.03 ± 0.80 A.U. in endometrial tumor tissue.

DTL expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between DTL primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of DTL and overall survival (OS) in patients with endometrial cancer, in white patients with low mutational burden (Figure 2). DTL primary tumor mRNA levels were a negative prognostic indicator in white endometrial cancer patients with low mutational burden, though median OS data do not reflect this. White patients with low mutational burden whose primary tumors expressed low levels of DTL possessed median OS of 103.73 months, while white patients with low mutational burden whose tumors expressed high levels of DTL possessed median OS of 112.17 months. This difference in OS based on DTL tumor expression in white patients with endometrial cancer with low mutational burden was statistically significant (Figure 2, Chart 3; logrank p -value: 0.02; hazard ratio: 2.28 (1.12-4.64)). DTL primary endometrial tumor expression was not correlated with overall survival in white patients with high mutational burden (Figure 2, Chart 3; logrank p -value: 0.85; hazard ratio: 1.08 (0.49-2.37)), nor in black patients with high ((Figure 2, Chart 3; logrank p -value: 0.41; hazard ratio: 2.01 (0.37-10.97)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.79; hazard ratio: 0.86 (0.29-2.58)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified denticleless E3 ubiquitin protein ligase homolog, encoded by DTL, as among the genes whose expression was most different, transcriptome-wide, in the endometrial tumor tissue of

1 patients with endometrial cancer when compared to benign endometrial tissue; we observed
2 significantly increased expression of DTL in endometrial tumor tissue as compared to benign
3 endometrial tissue. Further, we found a correlation between DTL expression and patient
4 survival outcomes in human endometrial cancer, as overall survival was generally inferior in
5 patients whose tumors expressed higher levels of DTL as compared to patients whose tumors
6 expressed lower levels of DTL, in white patients with low mutational burden, but not in white
7 patients with high mutational burden, nor in black patients with high or low mutational burden.

8 **Discussion**

9 We provided evidence here that denticleless E3 ubiquitin protein ligase homolog is among
10 the genes most differentially expressed in human endometrial cancer, that mRNA for DTL is
11 present at significantly increased quantity in endometrial tumor tissue as compared to benign
12 endometrium, and that DTL primary tumor expression is correlated with overall survival in white
13 endometrial cancer patients with low mutational burden. These data suggest DTL may be of
14 importance to fundamental biological processes that underlie the initiation, progression or
15 maintenance of human endometrial cancer.
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Rank: 184
Probe ID: 218585_s_at
p-value: 2.29E-04
t: -5.1541334
B: 0.86674
Gene: DTL
Gene name: denticleless E3 ubiquitin protein ligase homolog

Chart 1: DTL is differentially expressed in endometrial cancer when comparing primary endometrial tumors to benign endometrial tissue.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank: 274
probe ID: 222680_s_at
p-value: 2.42E-06
t: -5.0078555
B: 4.57468
Gene: DTL
Gene name: denticleless E3 ubiquitin protein ligase homolog

Chart 2: DTL is differentially expressed in endometrial cancer when comparing primary endometrial tumors to benign endometrial tissue.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 1: DTL is expressed at significantly higher levels in the endometrial tumors of patients with endometrial cancer when compared to benign endometrium.

The mRNA expression level of DTL in benign endometrial tissue (left) and in primary tumors of the endometrium (right) is graphically depicted; the result of a statistical test evaluating significance of difference in DTL expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0005$.

Figure 2: Correlation between DTL primary tumor expression and overall survival in endometrial cancer, in white patients with low mutational burden.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=543$ total endometrial cancer patients stratified into two groups, based on low or high expression of DTL in patient primary tumors, in black patients with high mutational burden (top left), black patients with low mutational burden (top right), white patients with high mutational burden (bottom left), and white patients with low mutational burden (bottom right). The log rank p -value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on DTL expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low DTL expression: 103.73 months

High DTL expression: 112.17 months

Chart 3: Median overall survival is superior in endometrial cancer patients with low primary tumor expression of DTL, in white patients with low mutational burden, though median OS data do not reflect this.

The median OS (overall survival) of white endometrial cancer patients with low mutational burden, with low primary tumor expression of DTL and high primary tumor expression of DTL is listed in this chart.