Over-expression of insulin degrading enzyme 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 the insulin degrading enzyme, encoded by IDE, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. IDE was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, primary tumor expression of IDE was correlated with overall survival in patients with endometrial cancer. IDE may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, IDE, insulin degrading enzyme, 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 insulin degrading enzyme (IDE) 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 IDE 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 IDE 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.

IDE is differentially expressed in endometrial cancer.

We identified the insulin degrading enzyme, encoded by IDE, 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, IDE ranked 5 out of 22273 transcripts, equating to 99.98% differential expression (Chart 1). Differential expression of IDE in human endometrial cancers was statistically significant (Chart 1; $p=5.49E-06$).

1 We queried a second microarray data to validate differential expression of IDE in
2 endometrial cancer. Again, we observed differential expression of IDE when comparing
3 endometrial tumor tissue to benign endometrial tissue (Chart 2). When sorting each of the genes
4 expressed in endometrial tumor tissue based on significance of change in expression as compared
5 to benign endometrial tissue, IDE ranked 335 out of 54675 transcripts, equating to 99.4%
6 differential expression (Chart 2). Differential expression of IDE in human endometrial cancers
7 trended towards statistical significance (Chart 2; $p=4.07E-06$).

8 **IDE is expressed at significantly higher levels in endometrial cancers as compared to benign** 9 **endometrial tissue.**

10 We obtained exact mRNA expression levels for IDE in endometrial tumor tissues and from
11 benign endometrial tissue to evaluate direction and statistical significance of change in expression
12 of IDE in human endometrial cancer. IDE was expressed at higher levels in endometrial tissue as
13 compared to normal endometrial tissue, and this difference was statistically significant (Figure 1;
14 $p<0.0001$). We calculated a mean fold change of 1.19 in IDE mRNA levels in human
15 endometrial cancer, as IDE was expressed at 6.54 ± 0.22 arbitrary units (A.U.) in control
16 endometrial tissue but at 7.79 ± 0.31 A.U. in endometrial tumor tissue.

17 **IDE expression is correlated with patient survival outcomes in endometrial cancer.**

18 We performed Kaplan-Meier survival analysis to evaluate correlation between IDE
19 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We
20 observed a correlation between primary tumor expression of IDE and overall survival in patients
21 with endometrial cancer, in the lower survival quartile (Figure 2). Patients whose primary tumors
22 expressed low levels of IDE possessed median OS of 41.63 months, while patients whose tumors
23 expressed high levels of IDE possessed median OS of 112.17 months. This difference in OS
24 based on IDE tumor expression in patients with endometrial cancer was statistically significant
25 (Figure 2, Chart 3; logrank p -value: 0.014; hazard ratio: 0.59 (0.38-0.9)).

26 Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we
27 identified insulin degrading enzyme, encoded by IDE, as among the genes whose expression was
28 most different, transcriptome-wide, in the endometrial tumor tissue of patients with endometrial
cancer when compared to benign endometrial tissue; we observed significantly increased
expression of IDE in endometrial tumor tissue as compared to benign endometrial tissue.
Further, we found a correlation between IDE expression and patient survival outcomes in human
endometrial cancer, as overall survival was superior in patients whose tumors expressed higher
levels of IDE as compared to patients whose tumors expressed lower levels of IDE.

Discussion

We provided evidence here that the insulin degrading enzyme is among the genes most differentially expressed in human endometrial cancer, that mRNA for IDE is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that IDE primary tumor expression is correlated with overall survival in patients with endometrial cancer. These data suggest IDE may be of importance to fundamental biological processes that underlie the initiation, progression or maintenance of human endometrial cancer.

References

1. Amant, F., Moerman, P., Neven, P., Timmerman, D., Van Limbergen, E. and Vergote, I., 2005. Endometrial cancer. *The Lancet*, 366(9484), pp.491-505.
2. Sorosky, J.I., 2008. Endometrial cancer. *Obstetrics & Gynecology*, 111(2), pp.436-447.
3. Morice, P., Leary, A., Creutzberg, C., Abu-Rustum, N. and Darai, E., 2016. Endometrial cancer. *The Lancet*, 387(10023), pp.1094-1108.
4. Sorosky, J.I., 2012. Endometrial cancer. *Obstetrics & Gynecology*, 120(2 Part 1), pp.383-397.
5. Pappa, K.I., Polyzos, A., Jacob-Hirsch, J., Amariglio, N., Vlachos, G.D., Loutradis, D. and Anagnou, N.P., 2015. Profiling of discrete gynecological cancers reveals novel transcriptional modules and common features shared by other cancer types and embryonic stem cells. *PLoS One*, 10(11), p.e0142229.
6. Day, R.S., McDade, K.K., Chandran, U.R., Lisovich, A., Conrads, T.P., Hood, B.L., Kolli, V.K., Kirchner, D., Litzi, T. and Maxwell, G.L., 2011. Identifier mapping performance for integrating transcriptomics and proteomics experimental results. *BMC bioinformatics*, 12(1), p.213.
7. Nagy, A., Munkacsy, G. and Gyorffy, B., 2020. Pancancer survival analysis of cancer hallmark genes. *bioRxiv*.

Rank: 5
Probe ID: 203328_x_at
p-value: 5.49E-06
t: -7.6453939
B: 4.28115
Gene: IDE
Gene name: insulin degrading enzyme

Chart 1: IDE 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: 335
probe ID: 203328_x_at
p-value: 4.07E-06
t: -4.8815517
B: 4.09339
Gene: IDE
Gene name: insulin degrading enzyme

Chart 2: IDE 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: IDE 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 IDE 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 IDE expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p<0.0001$.

Figure 2: Correlation between IDE primary tumor expression and overall survival in patients with endometrial cancer.

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 IDE in patient primary tumors, in the lower survival quartile. 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 IDE 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 IDE expression: 41.63 months
High IDE expression: 112.17 months

Chart 3: Median overall survival is superior in endometrial cancer patients with high primary tumor expression of IDE.

The median OS (overall survival) of patients with low primary tumor expression of IDE and high primary tumor expression of IDE is listed in this chart, for $n=543$ endometrial cancer patients, in the lower survival quartile.