Over-expression of ORMDL sphingolipid biosynthesis regulator 2 in human endometrial cancer.

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Gynecologic cancers including cancers of the endometrium are a clinical problem[1-4]. We mined published and public 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 ORMDL sphingolipid biosynthesis regulator 2, encoded by ORMDL2, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. ORMDL2 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of ORMDL2 was correlated with overall survival in white patients with low mutational burden. ORMDL2 may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, ORMDL2, ORMDL sphingolipid biosynthesis regulator 2, systems biology of endometrial cancer, targeted therapeutics in endometrial cancer.

Endometrial cancer is the most common gynecologic cancer in the developed world[1]. 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 integrative microarray dataset analysis, published and public[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 ORMDL sphingolipid biosynthesis regulator 2 (ORMDL2) in human endometrial cancer.

Methods

We utilized datasets GSE63678[5] and GSE39099[6] 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. GSE39099 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with *n*=2 benign endometrial tissues (*n*=1 normal endometrium and *n*=1 atypical endometrium) and *n*=2 endometrial cancers (*n*=1 early stage endometrial cancer and *n*=1 advanced stage endometrial cancer); 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 ORMDL2 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 tool[7] for correlation of ORMDL2 mRNA expression levels with overall survival in *n*=543 endometrial cancer patients.

Results

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

ORMDL2 is differentially expressed in endometrial cancer.

We identified ORMDL sphingolipid biosynthesis regulator 2, encoded by ORMDL2, 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, ORMDL2 ranked 165 out of 22273 transcripts, equating to 99.3% differential expression

(Chart 1). Differential expression of ORMDL2 in human endometrial cancers was statistically significant (Chart 1; p=1.88E-04).

We queried a second microarray data to validate differential expression of ORMDL2 in endometrial cancer. Again, we observed differential expression of ORMDL2 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, ORMDL2 ranked 6874 out of 54675 transcripts, equating to 87.4% differential expression (Chart 2). Differential expression of ORMDL2 in human endometrial cancers was not deemed statistically significant (Chart 2; p=0.102555).

ORMDL2 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for ORMDL2 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of ORMDL2 in human endometrial cancer. ORMDL2 was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; p=0.0018). We calculated a mean fold change of 1.17 in ORMDL2 mRNA levels in human endometrial cancer, as ORMDL2 was expressed at 8.17 $\pm$0.56 arbitrary units (A.U.) in control endometrial tissue but at 9.59 $\pm$0.44 A.U. in endometrial tumor tissue.

ORMDL2 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between ORMDL2 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of ORMDL2 and overall survival in patients with endometrial cancer, in white patients with low mutational burden (Figure 2). ORMDL2 primary tumor mRNA levels were a positive prognostic indicator in white endometrial cancer patients with low mutational burden. White patients with low mutational burden whose primary tumors expressed low levels of ORMDL2 possessed, on average, markedly shorter median OS as compared to white patients with low mutational burden whose tumors expressed high levels of ORMDL2 (Figure 2). This difference in OS based on ORMDL2 tumor expression in white patients with endometrial cancer with low mutational burden was statistically significant (Figure 2, Chart 3; logrank p-value: 0.027; hazard ratio: 0.46 (0.22-0.93)). ORMDL2 primary endometrial tumor expression was not correlated with overall survival in white patients with high mutational burden (Figure 2, Chart 3; logrank p-value: 0.82; hazard ratio: 0.91 (0.41-2.02)), nor in black patients with high ((Figure 2, Chart 3; logrank p-value: 0.28; hazard ratio: 0.4 (0.07-2.22)) or low mutational burden (Figure 2, Chart 3; logrank p-value: 0.57; hazard ratio: 0.73 (0.24-2.18)).

Thus, by mining published and public microarray data[5,6] in an unbiased and systematic fashion, we identified ORMDL sphingolipid biosynthesis regulator 2, encoded by ORMDL2, as among the genes whose expression was 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 ORMDL2 in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between ORMDL2 expression and patient survival outcomes in human endometrial cancer, as overall survival was superior in patients whose tumors expressed higher levels of ORMDL2 as compared to patients whose tumors expressed lower levels of ORMDL2, in white patients with low mutational burden, but not in white patients with high mutational burden, nor in black patients with high or low mutational burden.

Discussion

We provided evidence here that ORMDL sphingolipid biosynthesis regulator 2 is among the genes most differentially expressed in human endometrial cancer, that mRNA for ORMDL2 is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that ORMDL2 primary tumor expression is correlated with overall survival in white endometrial cancer patients with low mutational burden. These data suggest ORMDL2 may be of importance to fundamental biological processes that underlie the initiation, progression or maintenance of human endometrial cancer.

References

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Rank: 165
Probe ID: 218556_at
p-value: 1.88E-04
t: -5.2721541
B: 1.05096
Gene: ORMDL2
Gene name: ORMDL sphingolipid biosynthesis regulator 2

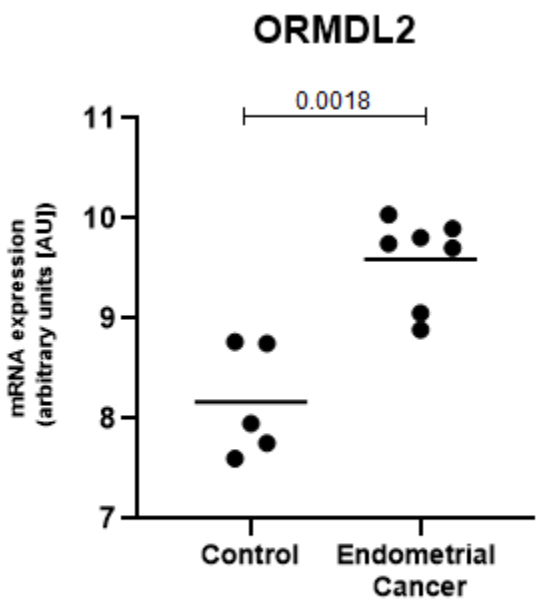
Chart 1: ORMDL2 is differentially expressed in endometrial cancer when comparing primary endometrial tumors to benign endometrial tissue.

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: 6874
probe ID: 218556_at
p-value: 0.102555
t: -2.0408178
B: -4.06
Gene: ORMDL2
Gene name: ORMDL sphingolipid biosynthesis regulator 2

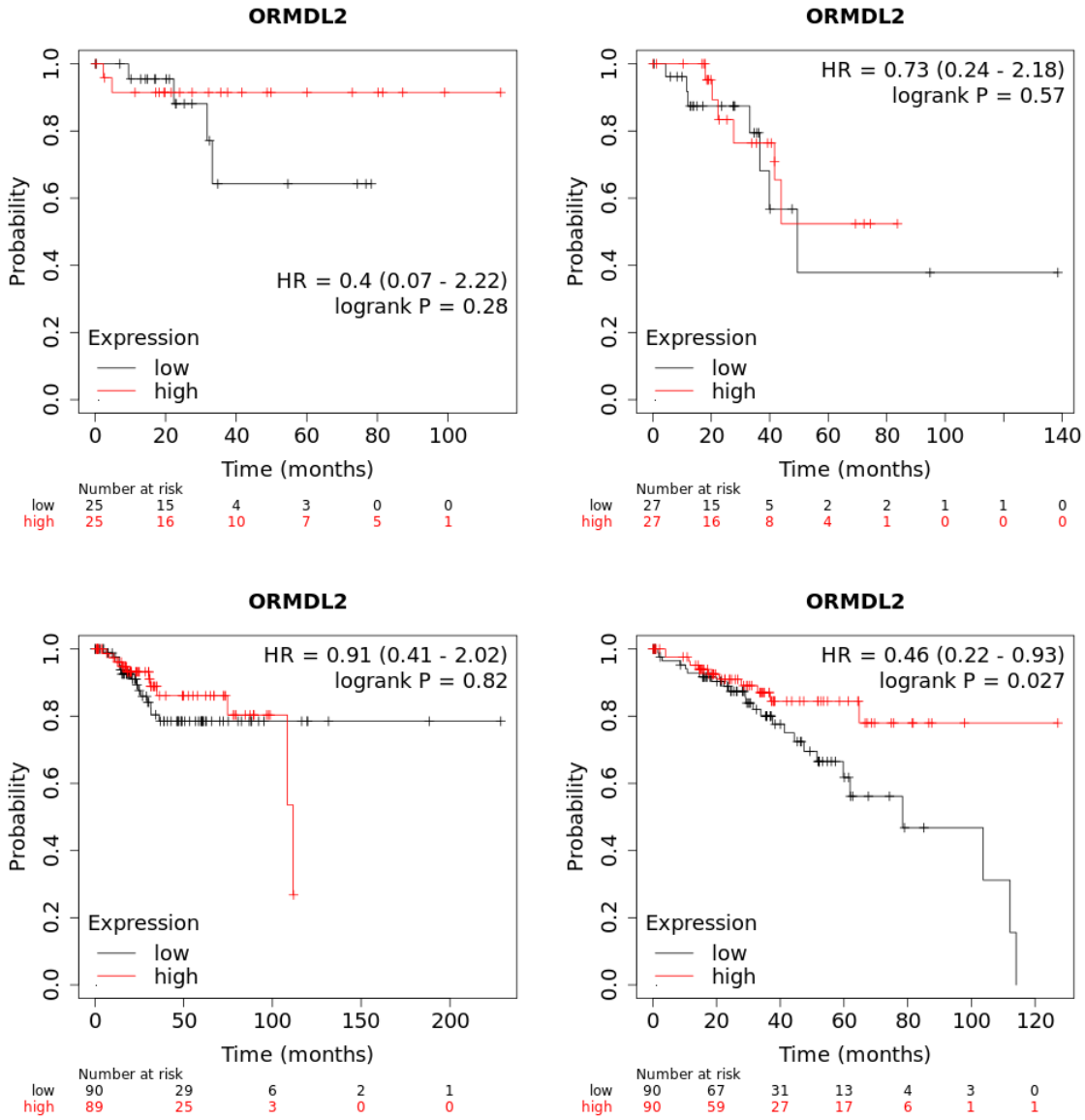
Chart 2: ORMDL2 is differentially expressed in endometrial cancer when comparing primary endometrial tumors to benign endometrial tissue.

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.



<u>Figure 1:</u> ORMDL2 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 ORMDL2 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 ORMDL2 expression between benign endometrial tissue and primary tumors of the endometrial tissue is p=0.0018



<u>Figure 2:</u> Correlation between ORMDL2 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 ORMDL2 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 ORMDL2 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.