Over-expression of NME/NM23 nucleoside diphosphate kinase 1 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 NME/NM23 nucleoside diphosphate kinase 1, encoded by NME1, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. NME1 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of NME1 was correlated with overall survival in white patients with high mutational burden. NME1 may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, NME1, NME/NM23 nucleoside diphosphate kinase 1, 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 NME/NM23 nucleoside diphosphate kinase 1 (NME1) 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 NME1 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 NME1 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.

NME1 is differentially expressed in endometrial cancer.

We identified NME/NM23 nucleoside diphosphate kinase 1, encoded by NME1, 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, NME1 ranked 162 out of 22273 transcripts, equating to 99.3% differential expression (Chart 1). Differential expression of NME1 in human endometrial cancers was statistically significant (Chart 1; $p=1.84E-04$).

We queried a second microarray data to validate differential expression of NME1 in endometrial cancer. Again, we observed differential expression of NME1 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, NME1 ranked 9737 out of 54675 transcripts, equating to 82.2% differential expression (Chart 2). Differential expression of NME1 in human endometrial cancers was statistically significant (Chart 2; $p=4.24E-02$).

NME1 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for NME1 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of NME1 in human endometrial cancer. NME1 was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0023$). We calculated a mean fold change of 1.18 in NME1 mRNA levels in human endometrial cancer, as NME1 was expressed at 9.39 ± 0.69 arbitrary units (A.U.) in control endometrial tissue but at 11.09 ± 0.51 A.U. in endometrial tumor tissue.

NME1 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between NME1 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of NME1 and overall survival in patients with endometrial cancer, in white patients with high mutational burden, in the lower survival quartile (Figure 2). NME1 primary tumor mRNA levels were a positive prognostic indicator in white endometrial cancer patients with high mutational burden. White patients with high mutational burden whose primary tumors expressed low levels of NME1 possessed median OS of 31.43 months, while white patients with high mutational burden whose tumors expressed high levels of NME1 possessed median OS of 111.63 months. This difference in OS based on NME1 tumor expression in white patients with endometrial cancer with high mutational burden was statistically significant (Figure 2, Chart 3; logrank p -value: 0.025; hazard ratio: 0.41 (0.19-0.92)). NME1 primary endometrial tumor expression was not correlated with overall survival in white patients with low mutational burden (Figure 2, Chart 3; logrank p -value: 0.7; hazard ratio: 0.86 (0.4-1.85)), nor in black patients with high (Figure 2, Chart 3; logrank p -value: 0.47; hazard ratio: 0.54 (0.1-2.96)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.34; hazard ratio: 2.05 (0.45-9.28)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified NME/NM23 nucleoside diphosphate kinase 1, encoded by NME1, 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 NME1 in endometrial tumor tissue as compared to benign
3 endometrial tissue. Further, we found a correlation between NME1 expression and patient
4 survival outcomes in human endometrial cancer, as overall survival was superior in patients
5 whose tumors expressed higher levels of NME1 as compared to patients whose tumors expressed
6 lower levels of NME1, in white patients with high mutational burden, but not in white patients
7 with low mutational burden, nor in black patients with high or low mutational burden.

8 **Discussion**

9 We provided evidence here that NME/NM23 nucleoside diphosphate kinase 1 is among the
10 genes most differentially expressed in human endometrial cancer, that mRNA for NME1 is
11 present at significantly increased quantity in endometrial tumor tissue as compared to benign
12 endometrium, and that NME1 primary tumor expression is correlated with overall survival in
13 white endometrial cancer patients with high mutational burden. These data suggest NME1 may
14 be of importance to fundamental biological processes that underlie the initiation, progression or
15 maintenance of human endometrial cancer.
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Rank: 162
Probe ID: 201577_at
p-value: 1.84E-04
t: -5.2864289
B: 1.0731
Gene: NME1
Gene name: NME/NM23 nucleoside diphosphate kinase 1

Chart 1: NME1 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: 9737
probe ID: 201577_at
p-value: 4.24E-02
t: -2.0565172
B: -4.21433
Gene: NME1
Gene name: NME/NM23 nucleoside diphosphate kinase 1

Chart 2: NME1 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: NME1 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 NME1 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 NME1 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0023$.

Figure 2: Correlation between NME1 primary tumor expression and overall survival in endometrial cancer, in white patients with high white 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 NME1 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), 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 NME1 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 NME1 expression: 31.43 months
High NME1 expression: 111.63 months

Chart 3: Median overall survival is superior in endometrial cancer patients with high primary tumor expression of NME1, in white patients with high mutational burden.

The median OS (overall survival) of white endometrial cancer patients with high mutational burden, with low primary tumor expression of NME1 and high primary tumor expression of NME1 is listed in this chart, in the lower survival quartile.