Over-expression of keratin 18 in human endometrial cancer.

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Gynecologic cancers including cancers of the endometrium are a clinical problem¹⁻⁴. 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 keratin 18, encoded by KRT18, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. KRT18 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of KRT18 was correlated with overall survival in white patients with high and low mutational burden. KRT18 may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, KRT18, keratin 18, 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 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 keratin 18 (KRT18) in human endometrial cancer.

Methods

We utilized datasets GSE63678⁵ and GSE39099⁶ 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 KRT18 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 KRT18 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.

KRT18 is differentially expressed in endometrial cancer.

We identified keratin 18, encoded by KRT18, 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, KRT18 ranked 110 out of 22273 transcripts, equating to 99.5% differential expression (Chart 1). Differential expression of KRT18 in human endometrial cancers was statistically significant (Chart 1; $p=1.09E-04$).

We queried a second microarray data to validate differential expression of KRT18 in endometrial cancer. Again, we observed differential expression of KRT18 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, KRT18 ranked 5885 out of 54674 transcripts, equating to 89.2% differential expression (Chart 2). Differential expression of KRT18 in human endometrial cancers trended towards statistical significance (Chart 2; $p=0.085815$).

KRT18 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for KRT18 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of KRT18 in human endometrial cancer. KRT18 was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0090$). We calculated a mean fold change of 1.35 in KRT18 mRNA levels in human endometrial cancer, as KRT18 was expressed at 9.30 ± 1.59 arbitrary units (A.U.) in control endometrial tissue but at 12.51 ± 0.48 A.U. in endometrial tumor tissue.

KRT18 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between KRT18 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of KRT18 and overall survival in patients with endometrial cancer, in white patients with high mutational burden (Figure 2). KRT18 primary tumor mRNA levels were a positive prognostic indicator in white endometrial cancer patients with high mutational burden, though median OS data do not reflect this due to changes in OS trends at probability=0.5. White patients with high mutational burden whose primary tumors expressed low levels of KRT18 possessed median OS of 111.63 months, while white patients with high mutational burden whose tumors expressed high levels of KRT18 possessed median OS of 108.37 months. This difference in OS based on KRT18 tumor expression in white patients with endometrial cancer with high mutational burden approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.092; hazard ratio: 0.5 (0.22-1.14)). We also observed a correlation between primary tumor expression of KRT18 and overall survival in patients with endometrial cancer, in white patients with low mutational burden (Figure 2). KRT18 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 KRT18 possessed median OS of 37.57 months, while white patients with low mutational burden whose tumors expressed high levels of KRT18 possessed median OS of 64.8 months. This difference in OS based on KRT18 tumor expression in white patients with endometrial cancer with low mutational burden was statistically

significant (Figure 2, Chart 3; logrank *p*-value: 0.044; hazard ratio: 0.49 (0.24-1)). KRT18 primary endometrial tumor expression was not correlated with overall survival in black patients with high ((Figure 2, Chart 3; logrank *p*-value: 0.37; hazard ratio: 2.15 (0.39-11.83)) or low mutational burden (Figure 2, Chart 3; logrank *p*-value: 0.76; hazard ratio: 1.19 (0.39-3.67)).

Thus, by mining published and public microarray data^{5,6} in an unbiased and systematic fashion, we identified keratin 18, encoded by KRT18, 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 KRT18 in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between KRT18 expression and patient survival outcomes in human endometrial cancer, as overall survival was generally superior in patients whose tumors expressed higher levels of KRT18 as compared to patients whose tumors expressed lower levels of KRT18, in white patients with high and low mutational burden, but not in black patients with high or low mutational burden.

Discussion

We provided evidence here that keratin 18 is among the genes most differentially expressed in human endometrial cancer, that mRNA for KRT18 is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that KRT18 primary tumor expression is correlated with overall survival in white endometrial cancer patients with high or low mutational burden. These data suggest KRT18 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: 110
Probe ID: 201596_x_at
p-value: 1.09E-04
t: -5.6059545
B: 1.55997
Gene: KRT18
Gene name: keratin 18

Chart 1: KRT18 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: 5885
probe ID: 201596_x_at
p-value: 0.085815
t: -2.1886043
B: -3.94
Gene: KRT18
Gene name: keratin 18

Chart 2: KRT18 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: KRT18 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 KRT18 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 KRT18 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0090$.

Figure 2: Correlation between KRT18 primary tumor expression and overall survival in endometrial cancer, in white patients with high and 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 KRT18 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 KRT18 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 KRT18 expression: 37.57 months

High KRT18 expression: 64.8 months

Chart 3: Median overall survival is superior in endometrial cancer patients with high primary tumor expression of KRT18, in white patients with low mutational burden.

The median OS (overall survival) of white endometrial cancer patients with low mutational burden, with low primary tumor expression of KRT18 and high primary tumor expression of KRT18 is listed in this chart.