Over-expression of BUB1 mitotic checkpoint serine/threonine kinase B 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 BUB1 mitotic checkpoint serine/threonine kinase B, encoded by BUB1B, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. BUB1B was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of BUB1B was correlated with recurrence-free survival in white patients with low mutational burden. BUB1B may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, BUB1B, BUB1 mitotic checkpoint serine/threonine kinase B, 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 BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B) 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 BUB1B 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 BUB1B mRNA expression levels with recurrence-free 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.

BUB1B is differentially expressed in endometrial cancer.

We identified BUB1 mitotic checkpoint serine/threonine kinase B, encoded by BUB1B, 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, BUB1B ranked 62 out of 22273 transcripts, equating to 99.7% differential expression (Chart 1). Differential expression of BUB1B in human endometrial cancers was statistically significant (Chart 1; $p=5.28E-05$).

We queried a second microarray data to validate differential expression of BUB1B in endometrial cancer. Again, we observed differential expression of BUB1B 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, BUB1B ranked 453 out of 54675 transcripts, equating to 99.2% differential expression (Chart 2). Differential expression of BUB1B in human endometrial cancers was statistically significant (Chart 2; $p=1.11\text{E-}05$).

BUB1B is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for BUB1B in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of BUB1B in human endometrial cancer. BUB1B was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0002$). We calculated a mean fold change of 1.42 in BUB1B mRNA levels in human endometrial cancer, as BUB1B was expressed at 6.19 ± 0.39 arbitrary units (A.U.) in control endometrial tissue but at 8.81 ± 0.98 A.U. in endometrial tumor tissue.

BUB1B expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between BUB1B primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of BUB1B and recurrence-free survival in patients with endometrial cancer, in white patients with low mutational burden (Figure 2). BUB1B primary tumor mRNA levels were a negative prognostic indicator in white endometrial cancer patients with low mutational burden. White patients with low mutational burden whose primary tumors expressed low levels of BUB1B possessed, on average, markedly greater median RFS as compared to white patients with low mutational burden whose tumors expressed high levels of BUB1B (Figure 2). This difference in RFS based on BUB1B tumor expression in white patients with endometrial cancer with low mutational burden was statistically significant (Figure 2, Chart 3; logrank p -value: 0.042; hazard ratio: 2.58 (1-6.66)). BUB1B primary endometrial tumor expression was not correlated with recurrence-free survival in white patients with high mutational burden (Figure 2, Chart 3; logrank p -value: 0.59; hazard ratio: 0.77 (0.3-1.96)), nor in black patients with high (Figure 2, Chart 3; logrank p -value: 0.61; hazard ratio: 1.58 (0.26-9.45)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.78; hazard ratio: 1.23 (0.29-5.25)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified BUB1 mitotic checkpoint serine/threonine kinase B, encoded by BUB1B, 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 BUB1B in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between BUB1B expression and patient survival outcomes in human endometrial cancer, as recurrence-free survival was inferior in patients whose tumors expressed higher levels of BUB1B as compared to patients whose tumors expressed lower levels of BUB1B, 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 BUB1 mitotic checkpoint serine/threonine kinase B is among the genes most differentially expressed in human endometrial cancer, that mRNA for BUB1B is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that BUB1B primary tumor expression is correlated with recurrence-free survival in white endometrial cancer patients with low mutational burden. These data suggest BUB1B 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: 62
Probe ID: 203755_at
p-value: 5.28E-05
t: -6.0684016
B: 2.23525
Gene: BUB1B
Gene name: BUB1 mitotic checkpoint serine/threonine kinase B

Chart 1: BUB1B 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: 453
probe ID: 203755_at
p-value: 1.11E-05
t: -4.6317702
B: 3.16341
Gene: BUB1B
Gene name: BUB1 mitotic checkpoint serine/threonine kinase B

Chart 2: BUB1B 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: BUB1B 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 BUB1B 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 BUB1B expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0002$.

Figure 2: Correlation between BUB1B primary tumor expression and recurrence-free survival in endometrial cancer, in white patients with low mutational burden.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=543$ total endometrial cancer patients stratified into two groups, based on low or high expression of BUB1B 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 recurrence-free 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 BUB1B 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.