Over-expression of tubulin beta 4B class IVb 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 tubulin beta 4B class IVb, encoded by TUBB4B, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. TUBB4B was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, primary tumor expression of TUBB4B was correlated with overall survival in patients with endometrial cancer. TUBB4B may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, TUBB4B, tubulin beta 4B class IVb, 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 tubulin beta 4B class IVb (TUBB4B) 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 TUBB4B 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 TUBB4B 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.

TUBB4B is differentially expressed in endometrial cancer.

We identified tubulin beta 4B class IVb, encoded by TUBB4B, 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, TUBB4B ranked 33 out of 22273 transcripts, equating to 99.9% differential expression (Chart 1). Differential expression of TUBB4B in human endometrial cancers was statistically significant (Chart 1;

$p=2.53\text{E-}05$).

We queried a second microarray data to validate differential expression of TUBB4B in endometrial cancer. Again, we observed differential expression of TUBB4B 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, TUBB4B ranked 2100 out of 54675 transcripts, equating to 96.2% differential expression (Chart 2). Differential expression of TUBB4B in human endometrial cancers trended towards statistical significance (Chart 2; $p=9.53\text{E-}04$).

TUBB4B is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for TUBB4B in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of TUBB4B in human endometrial cancer. TUBB4B was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 1.10 in TUBB4B mRNA levels in human endometrial cancer, as TUBB4B was expressed at 10.46 ± 0.18 arbitrary units (A.U.) in control endometrial tissue but at 11.54 ± 0.33 A.U. in endometrial tumor tissue.

TUBB4B expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between TUBB4B primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of TUBB4B and overall survival (RFS) in patients with endometrial cancer, in the lower survival quartile (Figure 2). Patients whose primary tumors expressed low levels of TUBB4B possessed median RFS of 47.6 months as compared to patients whose tumors expressed high levels of TUBB4B (Figure 2). This difference in RFS based on TUBB4B tumor expression in patients with endometrial cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.0093; hazard ratio: 0.57 (0.38-0.88)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified tubulin beta 4B class IVb, encoded by TUBB4B, 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 TUBB4B in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between TUBB4B expression and patient survival outcomes in human endometrial cancer, as overall survival was inferior in patients whose tumors expressed higher levels of TUBB4B as compared to patients whose tumors expressed lower

levels of TUBB4B.

Discussion

We provided evidence here that tubulin beta 4B class IVb is among the genes most differentially expressed in human endometrial cancer, that mRNA for TUBB4B is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that TUBB4B primary tumor expression is correlated with overall survival in patients with endometrial cancer. These data suggest TUBB4B 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: 33

Probe ID: 213726_x_at

p-value: 2.53E-05

t: -6.5560525

B: 2.90966

Gene: TUBB4B

Gene name: tubulin beta 4B class IVb

Chart 1: TUBB4B 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: 2100

probe ID: 213726_x_at

p-value: 9.53E-04

t: -3.4067783

B: -0.90743

Gene: TUBB4B

Gene name: tubulin beta 4B class IVb

Chart 2: TUBB4B 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.

TUBB4B

12.5

<0.0001

Figure 1: TUBB4B 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 TUBB4B 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 TUBB4B expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p < 0.0001$.

Figure 2: Correlation between TUBB4B 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 TUBB4B in patient primary tumors. 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 TUBB4B 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 TUBB4B expression: 47.6 months

High TUBB4B expression: 114.1 months

Chart 3: Median overall survival is superior in endometrial cancer patients with high primary tumor expression of TUBB4B.

The median OS (overall survival) of patients with low primary tumor expression of TUBB4B and high primary tumor expression of TUBB4B is listed in this chart, for $n=543$ endometrial cancer patients.