Over-expression of structural maintenance of chromosomes 4 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 structural maintenance of chromosomes 4, encoded by SMC4, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. SMC4 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, primary tumor expression of SMC4 was correlated with overall survival in patients with endometrial cancer. SMC4 may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, SMC4, structural maintenance of chromosomes 4, 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 structural maintenance of chromosomes 4 (SMC4) 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 SMC4 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 SMC4 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.

SMC4 is differentially expressed in endometrial cancer.

We identified structural maintenance of chromosomes 4, encoded by SMC4, 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, SMC4 ranked 3 out of 22273 transcripts, equating to 99.99% differential expression (Chart 1). Differential expression of SMC4 in human endometrial cancers was statistically significant (Chart 1; $p=1.96E-06$).

We queried a second microarray data to validate differential expression of SMC4 in endometrial cancer. Again, we observed differential expression of SMC4 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, SMC4 ranked 758 out of 54675 transcripts, equating to 98.6% differential expression (Chart 2). Differential expression of SMC4 in human endometrial cancers trended towards statistical significance (Chart 2; $p=5.16E-05$).

SMC4 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for SMC4 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of SMC4 in human endometrial cancer. SMC4 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 1.15 in SMC4 mRNA levels in human endometrial cancer, as SMC4 was expressed at 9.02 ± 0.20 arbitrary units (A.U.) in control endometrial tissue but at 10.42 ± 0.32 A.U. in endometrial tumor tissue.

SMC4 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between SMC4 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of SMC4 and overall survival in patients with endometrial cancer (Figure 2). Patients whose primary tumors expressed low levels of SMC4 possessed median OS of 108.37 months, while patients whose tumors expressed high levels of SMC4 possessed median OS of 37.57 months. This difference in OS based on SMC4 tumor expression in patients with endometrial cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.001; hazard ratio: 2.03 (1.32-3.12)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified structural maintenance of chromosomes 4, encoded by SMC4, 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 SMC4 in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between SMC4 expression and patient survival outcomes in human endometrial cancer, as overall survival was inferior in patients whose tumors expressed higher levels of SMC4 as compared to patients whose tumors expressed lower levels of SMC4.

Discussion

We provided evidence here that structural maintenance of chromosomes 4 is among the genes most differentially expressed in human endometrial cancer, that mRNA for SMC4 is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that SMC4 primary tumor expression is correlated with overall survival in patients with endometrial cancer. These data suggest SMC4 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: 3
Probe ID: 201664_at
p-value: 1.96E-06
t: -8.4422152
B: 5.1742
Gene: SMC4
Gene name: structural maintenance of chromosomes 4

Chart 1: SMC4 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: 758
probe ID: 201663_s_at
p-value: 5.16E-05
t: -4.2344747
B: 1.74871
Gene: SMC4
Gene name: structural maintenance of chromosomes 4

Chart 2: SMC4 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: SMC4 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 SMC4 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 SMC4 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p < 0.0001$.

Figure 2: Correlation between SMC4 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 SMC4 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 SMC4 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 SMC4 expression: 108.37 months

High SMC4 expression: 37.57 months

Chart 3: Median overall survival is superior in endometrial cancer patients with low primary tumor expression of SMC4.

The median OS (overall survival) of patients with low primary tumor expression of SMC4 and high primary tumor expression of SMC4 is listed in this chart, for $n=543$ endometrial cancer patients.