Over-expression of MLX, MAX dimerization protein in human endometrial cancer.

Shahan Mamoor, MS¹
¹shahanmamoor@gmail.com
East Islip, NY USA

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 MLX, MAX dimerization protein, encoded by MLX, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. MLX was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of MLX was correlated with recurrence-free survival in black patients with low mutational burden. MLX may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, MLX, MLX, MAX dimerization protein, 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 MLX, MAX dimerization protein (MLX) 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 MLX 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 MLX 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.

MLX is differentially expressed in endometrial cancer.

We identified MLX, MAX dimerization protein, encoded by MLX, 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, MLX ranked 130 out of 22273 transcripts, equating to 99.4% differential expression (Chart 1). Differential expression of MLX in human endometrial cancers was statistically significant (Chart 1; $p=1.29E-04$).

We queried a second microarray data to validate differential expression of MLX in endometrial cancer. Again, we observed differential expression of MLX 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, MLX ranked 6951 out of 54675 transcripts, equating to 87.3% differential expression (Chart 2). Differential expression of MLX in human endometrial cancers was statistically significant (Chart 2; $p=2.06E-02$).

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

We obtained exact mRNA expression levels for MLX in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of MLX in human endometrial cancer. MLX 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 1.07 in MLX mRNA levels in human endometrial cancer, as MLX was expressed at 8.73 ± 0.16 arbitrary units (A.U.) in control endometrial tissue but at 9.38 ± 0.18 A.U. in endometrial tumor tissue.

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

We performed Kaplan-Meier survival analysis to evaluate correlation between MLX primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of MLX and recurrence-free survival in patients with endometrial cancer, in black patients with low mutational burden, in the lower survival quartile (Figure 2). MLX primary tumor mRNA levels were a positive prognostic indicator in black endometrial cancer patients with low mutational burden. Black patients with low mutational burden whose primary tumors expressed low levels of MLX possessed median RFS of 16.83 months, while black patients with low mutational burden whose tumors expressed high levels of MLX possessed median RFS of 48.43 months. This difference in RFS based on MLX tumor expression in black patients with endometrial cancer with low mutational burden approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.043; hazard ratio: 0.25 (0.06-1.06)). MLX primary endometrial tumor expression was not correlated with recurrence-free survival in black patients with high mutational burden (Figure 2, Chart 3; logrank p -value: 0.82; hazard ratio: 0.81 (0.13-4.85)), nor in white patients with high ((Figure 2, Chart 3; logrank p -value: 0.68; hazard ratio: 1.27 (0.42-3.85)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.48; hazard ratio: 0.72 (0.29-1.79)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified MLX, MAX dimerization protein, encoded by MLX, as among the genes whose expression was most different, transcriptome-wide, in the endometrial tumor tissue of patients

1 with endometrial cancer when compared to benign endometrial tissue; we observed
2 significantly increased expression of MLX in endometrial tumor tissue as compared to benign
3 endometrial tissue. Further, we found a correlation between MLX expression and patient
4 survival outcomes in human endometrial cancer, as recurrence-free survival was superior in
5 patients whose tumors expressed higher levels of MLX as compared to patients whose tumors
6 expressed lower levels of MLX, in black patients with low mutational burden, but not in black
7 patients with high mutational burden, nor in white patients with high or low mutational burden.

8 **Discussion**

9 We provided evidence here that MLX, MAX dimerization protein is among the genes most
10 differentially expressed in human endometrial cancer, that mRNA for MLX is present at
11 significantly increased quantity in endometrial tumor tissue as compared to benign endometrium,
12 and that MLX primary tumor expression is correlated with recurrence-free survival in black
13 endometrial cancer patients with low mutational burden. These data suggest MLX may be of
14 importance to fundamental biological processes that underlie the initiation, progression or
15 maintenance of human endometrial cancer.
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Rank: 130
Probe ID: 213708_s_at
p-value: 1.29E-04
t: -5.504246
B: 1.40678
Gene: MLX
Gene name: MLX, MAX dimerization protein

Chart 1: MLX 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: 6951
probe ID: 1570347_at
p-value: 2.06E-02
t: -2.3539092
B: -3.60862
Gene: MLX
Gene name: MLX, MAX dimerization protein

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

Figure 2: Correlation between MLX primary tumor expression and recurrence-free survival in endometrial cancer, in black 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 MLX 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 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 MLX 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 MLX expression: 16.83 months

High MLX expression: 48.43 months

Chart 3: Median recurrence-free survival is superior in endometrial cancer patients with high primary tumor expression of MLX, in black patients with low mutational burden.

The median RFS (recurrence-free survival) of black endometrial cancer patients with low mutational burden, with low primary tumor expression of MLX and high primary tumor expression of MLX is listed in this chart, in the lower survival quartile.