Differential expression of MAZ in cancers of the breast.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

Breast cancer affects women at relatively high frequency¹. We mined published microarray datasets^{2,3} to determine in an unbiased fashion and at the systems level genes most differentially expressed in the primary tumors of patients with breast cancer. We identified significant differential expression of the MYC-associated zinc finger protein and transcription factor MAZ when comparing primary tumors of the breast to the tissue of origin, the normal breast. MAZ may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, MAZ, Myc-associated zinc finger protein, systems biology of breast cancer, targeted therapeutics in breast cancer.

Invasive breast cancer is diagnosed in over a quarter of a million women in the United States each year¹ and in 2018, breast cancer was the leading cause of cancer death in women worldwide⁴. While patients with localized breast cancer are provided a 99% 5-year survival rate, patients with regional breast cancer, cancer that has spread to lymph nodes or nearby structures, are provided an 86% 5-year survival rate^{5,6}. Patients with metastasis to distant sites are provided a 27% 5-year survival rate^{5,6}. Understanding how primary tumors are most transcriptionally different from the tissue from which they originate, the breast, can facilitate development of novel diagnostic and therapeutics to promote early detection and enhanced treatment, and contribute to efforts to prevent progression to metastatic stages. We mined published microarray data^{2,3} to understand at the transcriptome level and in a unbiased fashion genes most differentially expressed in primary tumors of the breast as compared to normal breast tissue. The transcription factor MAZ emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE109169² and GSE124648³ for this differential gene expression analysis of female breast cancer. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with n=25 normal breast tissue and n=25 tumors of the breast and; analysis was performed using platform GPL5175. The tissues from this first dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). GSE124648 was generated using Affymetrix Human Genome U133A Array technology with n=10 normal breast tissue and n=126 tumors of the breast and; analysis was performed using platform GPL96. 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 MAZ expression was significantly between primary breast tumors and breast tissue using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of MAZ mRNA expression levels with relapse-free survival (disease-free survival) in $n=3951$ breast cancer patients, in the upper and lower survival quartiles.

Results

We performed discovery of genes associated with breast cancer in females by mining two independently published microarray datasets^{2,3}.

MAZ is differentially expressed in primary tumors of the breast.

Comparison of 25 normal breast tissues to 25 tumors of the breast² revealed that the MYC-associated zinc finger MAZ was among the genes most differentially expressed in tumors of the breast in female breast cancer (Table 1). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, MAZ ranked 170 out of 19076 total transcripts, equating to 99.1% differential expression (Table 1). Differential expression of MAZ in female breast cancer was statistically significant (Table 1; $p=1.74E-13$).

Analysis of a second microarray dataset³, here in 126 tumors of the breast as compared

to 10 normal breast tissues, again revealed significant differential expression of MAZ in tumors of the breast in female breast cancer (Table 2). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, MAZ ranked 186 out of 22283 total transcripts, equating to 99.2% differential expression (Table 2). Differential expression of MAZ in female breast cancer was statistically significant (Table 2; $p=1.34E-14$).

MAZ is expressed at significantly higher levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for MAZ from the breast and from breast tumors to understand the magnitude and direction of MAZ expression change. In both datasets, MAZ was expressed at higher levels in tumors of the breast as compared to normal breast tissue (Figure 1 and Figure 2). Increased expression of MAZ in primary breast tumors was statistically significant (Figure 1: $p<0.0001$ and Figure 2: $p<0.0001$). We calculated a mean fold change of 1.1 ± 0.04 and 1.1158 ± 0.0392 when comparing primary tumors of the breast to normal breast tissues (Table 1 and Table 2; respectively).

MAZ expression may be associated with survival outcomes in female breast cancer.

We performed Kaplan-Meier survival analysis in 3951 breast cancer patients, in an attempt to correlate MAZ mRNA expression levels with patient survival. We found a modest but statistically significant correlation between relapse-free survival (also known as disease-free survival) and MAZ expression in breast tumors in patients of the lower but not upper survival quartile (Figure 3A and 3B; log rank p -value=0.75 for patients in the upper quartile (Fig. 3A) and

log rank p -value=0.0024, and hazard ratio 0.83 (0.73 - 0.94) for patients in the lower quartile (Fig 3B)). For patients in the lower survival quartile, median RFS was 161.29 months for patients whose tumors expressed low levels of MAZ while median RFS was 216.66 months for patients whose tumors expressed high levels of MAZ (Table 3).

Thus, through comparative transcriptome analysis of primary tumors of the breast and normal breast tissue, we found that differential expression and up-regulation of MAZ was among the most significant transcriptional features in primary tumors from patients with breast cancer. In patients in the lower but not upper quartile, MAZ expression in primary tumors of the breast was correlated with relapse-free survival in patients with breast cancer, with higher expression of MAZ correlating with improved survival.

Discussion

Invasive breast cancer is medical problem with a 27% 5-year survival rate for women whose disease has spread to distant sites. To facilitate understanding of the basic transcriptional differences between primary tumors of the breast and the tissues from which these tumors originate, normal breast tissues, we performed comparative transcriptome analysis using two independently published microarray datasets^{2,3} and identified significant differential expression of the Myc-associated zinc finger and transcription factor MAZ in female breast cancer.

MAZ is zinc-finger transcription factor belonging to the MYC family of proteins with trans-activating functions; structurally, MAZ contains six zinc fingers, multiple polyalanine tracts and an amino-terminal proline-rich domain⁸. Two promoters, termed P1 and P2 have previously been described to function as regulatory elements for transcription of MYC^{9,10}. MAZ can bind to

ME1a1, a regulatory sequence located between P1 and P2, at a GA box in the P2 promoter and at the C2 termination site⁸. Expression of MAZ was described in all tissues except for the kidney⁸. Its activity is regulated by the casein kinase II¹¹ and its phosphorylation by the protein kinase Akt can induce transcriptional activation of p53 by promoting release of MAZ from the p53 promoter¹².

MAZ has been implicated in a number of human cancers. In hepatocellular carcinoma (HCC), MAZ expression has been correlated with that of ZEB1 and ZEB2^{13,14}, expressed at higher levels in HCC tissues as compared to adjacent tissues, and its higher expression was correlated with worse survival outcomes in patients with HCC. MAZ expression promoted invasion and metastasis in SMMC-7221 HCC cells *in vitro*, tumor growth of SMMC-7221 cells *in vivo*, and induced expression of genes associated with the epithelial to mesenchymal transition (EMT)¹³. MAZ has also been described as over-expressed in pancreatic ductal adenocarcinoma, regulating expression of BIRC5¹⁵. In breast cancer, MAZ has been shown to be over-expressed in the MCF-7 breast cancer cell line, and over-expression of MAZ in human mammary epithelial cells could induce expression of the peroxisome proliferator-activated receptor gamma 1 (PPAR γ 1)¹⁶. A separate study argued that in basal-like breast cancer (BLBC), MAZ functioned both to promote proliferation of tumor cells but could inhibit cancer progression, and identified FOXF2 as a target of MAZ in BLBC¹⁷. Also known as the serum amyloid factor-1, or SAF-1, MAZ could occupy the promoter of the vascular endothelial growth factor (VEGF) and induce expression of VEGF in triple negative breast cancer cell line MDA-MB-231¹⁸. Thus, MAZ over-expression has been reported in multiple solid tumor types, but the mechanisms by which MAZ functions in human cancer and specifically in human breast cancer and how these functions might differ by breast cancer type are not completely clear.

1 We found significant differential expression and up-regulation of the Myc-associated
2 zinc-finger protein MAZ in human breast cancer across independent microarray datasets^{2,3}.
3 MAZ expression may be correlated with relapse-free survival in patients in the lower but not
4 upper survival quartile. Future studies can determine by which mechanisms MAZ functions to
5 contribute to breast cancer initiation or progression, and which of these are the most significant
6 with respect to driving aggressive behavior.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
170	3655665	1.74E-13	9.91	20.518538	1.1 ± 0.04	MAZ	MYC-associated zinc finger protein

Table 1: MAZ is differentially expressed in human breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, fold change of MAZ in primary breast tumors as compared to normal breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
186	212064_x_at	1.34E-14	8.635914	22.7265	1.1158 ± 0.0392	MAZ	MYC associated zinc finger protein

Table 2: MAZ is differentially expressed in human breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, fold change of MAZ in primary breast tumors as compared to normal breast, gene and gene name are listed in this chart.

Figure 1: MAZ is expressed at significantly higher levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of MAZ in normal breast tissue (left) and in primary tumors of the breast (right) is graphically depicted with the result of a statistical test evaluating significance of difference in MAZ expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.

Figure 2: MAZ is expressed at significantly higher levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of MAZ in normal breast tissue (left) and in primary tumors of the breast (right) is graphically depicted with the result of a statistical test evaluating significance of difference in MAZ expression between normal breast tissue and primary tumors of the breast, a p -value, listed above.

Figure 3: Modest correlation of MAZ with relapse-free survival in patients with breast cancer, in the lower but not upper survival quartile.

Depicted in this Kaplan-Meier plot is the probability of relapse-free survival (RFS) for $n=3951$ total patients stratified into two groups, based on low or high expression of MAZ in patient primary tumors. Analysis is performed for a, patients in the lower survival quartile (A), and patients in the upper survival quartile (B). The log rank p -value denoting statistical significance of difference in relapse-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 MAZ 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 expression cohort (months)	High expression cohort (months)
161.29	216.66

Table 3: Modest correlation of MAZ expression in breast tumors with relapse-free survival in patients in the lower quartile.

The relapse-free survival of $n=3951$ breast cancer patients based on stratification into low or high tumor expression of MAZ is listed in this chart, in patients of the lower quartile.