The accessory protein of the IL-18 receptor, IL-18 RAP, is differentially expressed and up-regulated in the bone metastases of patients with metastatic breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

To understand the transcriptional nature of metastasis to disparate sites in human breast cancer, we mined published microarray data^{1,2}, comparing global gene expression profiles of metastasis to the bones and to the lymph nodes. We discovered that the IL-18 receptor accessory protein, encoded by *IL18RAP*, was among the genes whose expression was most different, transcriptome-wide, in bone and lymph node metastases. IL18RAP mRNA was present at significantly higher quantities in metastasis to the bone as compared to metastasis to the lymph nodes. Analysis of patient survival data revealed that expression of IL18RAP in primary tumors of the breast was correlated with overall survival, in lymph node negative patients but not in lymph node positive patients. Signal transduction through the IL-18 receptor may be important in understanding basic differences between the transcriptional composition of metastasis to the lymph nodes and to the bones in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, bone metastasis, lymph node metastasis, IL18RAP, IL-18 receptor accessory protein, systems biology of breast cancer, targeted therapeutics in breast cancer, innate immunity in cancer.

Understanding how the transcriptional landscape of metastasis differs based on anatomical site can facilitate fundamental understanding of cancer progression in humans. We performed a global comparative analysis of metastatic tumor tissues in patients with metastatic breast cancer, comparing bone and lymph node metastases^{1,2}. We discovered transcriptome-wide differential expression of the gene encoding the IL-18 receptor accessory protein, IL18RAP, in the bone metastases of patients with metastatic breast cancer.

Methods

We used dataset GSE46141¹ and GSE124648² for this comparative transcriptome analysis of lymph node and bone metastasis in metastatic breast cancer in conjunction with GEO2R. GSE46141 was generated using Rosetta/Merck Human RSTA Custom Affymetrix 2.0 microarray technology, and this analysis compared $n=39$ lymph node metastatic tissues and $n=5$ bone metastatic tissues; analysis was performed using platform GPL10379. GSE124648 was generated using Affymetrix Human Genome U133A array technology with $n=44$ lymph node metastases and $n=11$ bone metastases from patients with breast cancer; analysis was performed using platform GPL96. 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 IL18RAP gene expression was significantly different between metastasis to the lymph nodes and the bones in humans with breast cancer using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool³ for correlation of IL18RAP mRNA expression levels with overall survival in $n=452$ lymph node positive patients and $n=726$ lymph node negative breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic tumor tissues of patients with metastatic breast cancer using published microarray data¹ to describe the transcriptional landscape of metastasis in human breast cancer in an unbiased fashion. In this study, we compared metastasis to the bones and to the lymph nodes in human breast cancer.

IL18RAP is differentially expressed in bone metastasis in human breast cancer.

Through blind, systems-level analysis of published microarray data¹, we identified The IL-18 receptor accessory protein encoded by IL18RAP, as among the genes whose expression was most significantly different, transcriptome-wide, in the bone metastatic tissues of humans with breast cancer when compared to lymph node metastasis (Table 1). When sorting each of the genes expressed in bone metastases based on significance of difference as compared to lymph node metastases in patients with breast cancer, IL18RAP ranked 34 out of 51565 total transcripts (Chart 1), equating to 99.9% differential expression. Differential expression of IL18RAP in the bone metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=3.29e-05$).

We queried a second microarray dataset² to attempt to validate transcriptome-wide differential expression of IL18RAP in metastatic tissues in human breast cancer. We again observed IL18RAP to be among the genes whose expression was most quantitatively different in metastasis to the bones in patients with metastatic breast cancer as compared to lymph node metastases (Chart 2). When sorting each of the genes expressed in bone metastases based on significance of difference as compared to lymph node metastases, IL18RAP ranked 605 out of 22283 total transcripts (Chart 2), equating to 97.3% differential expression. Differential expression of IL18RAP in the bone metastases of patients with metastatic breast

cancer was statistically significant (Chart 2; $p=5.55e-03$). Together, the data suggested that differential expression of IL-18 RAP in bone metastatic breast cancer may be a *bona fide* biological phenomena.

IL18RAP is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for IL18RAP, in bone and lymph node metastatic tissues in patients with metastatic breast cancer to determine direction and statistical significance of change in IL18RAP expression between metastasis to disparate sites. We observed significantly increased expression of IL18RAP in bone metastases of patients with breast cancer as compared to lymph node metastases: IL18RAP was expressed at 6.00 ± 1.13 arbitrary units (AU) in lymph node metastases, while it was expressed at 8.77 ± 2.33 AU in bone metastatic tissues (Figure 1). The difference in IL18RAP mRNA levels between metastasis to the bones and to the lymph nodes was statistically significant (Figure 1; $p=0.00005$).

IL18RAP expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis³ in $n=452$ lymph node positive breast cancer patients and $n=726$ lymph node negative breast cancer patients, to evaluate whether IL18RAP tumor expression was correlated with survival outcomes in breast cancer and to evaluate whether lymph node status influenced correlation of IL18RAP tumor expression with survival outcomes in human breast cancer. We observed a correlation between primary tumor expression of IL18RAP and overall survival (OS) in lymph node negative breast cancer, in the upper survival quartile (Figure 2). Lymph node negative patients whose primary tumors expressed low levels of IL18RAP possessed, on average, inferior median OS as compared to lymph node negative patients whose tumors expressed high levels of IL18RAP (Figure 2). This difference in OS based on IL18RAP tumor expression in lymph node negative patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.036; hazard ratio: 0.6 (0.37-0.97)). IL18RAP primary tumor expression was not correlated with overall survival outcomes in lymph node positive patients (Figure 2, Chart 3; logrank p -value: 0.63; hazard ratio: 0.91 (0.62-1.34)).

Thus, by mining published microarray data^{1,2} in an unbiased and systematic fashion, we identified IL18RAP as among the genes whose expression was most different, transcriptome-wide, in the bone metastases of patients with breast cancer when compared to metastasis to the lymph nodes; we observed significantly increased expression of IL18RAP in bone metastases. Further, we observed correlation between IL18RAP expression and overall survival in lymph node negative patients but not in lymph node positive patients.

Discussion

We provided evidence here that IL18RAP is among the genes whose expression is most quantitatively different when comparing global gene expression in metastasis to the bone and to the lymph nodes in human breast cancer, and that IL18RAP expression in primary tumors of the breast is correlated with overall survival in lymph node negative patients but not in lymph node positive patients. We recently demonstrated that two separate pattern recognition receptors required for defense against *Yersinia pestis* infection, or modulated during the course of *Yersinia pestis* infection⁴⁻⁶, NLRP12 and MEFV (PYRIN), are among the genes whose expression is most significantly different, transcriptome-wide, in bone metastasis in human breast cancer when compared to lymph node metastasis^{7,8}; found among a report establishing the role for NLRP12 in innate immune signal transduction during *Yersinia pestis* infection is a demonstration that the IL-18 signaling pathway is specifically important for defense against *Yersinia pestis* infection⁴. The data, in sum, argue for a critical evaluation of the presence of *Yersinia pestis* and *Yersinia*-like pathogens in human breast cancer.

References

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Rank: 34
Probe ID: 100141328_TGI_at
p-value: 3.29e-05
t: -4.62
B: 2.07593
Gene: IL18RAP
Gene name: interleukin 18 receptor accessory protein

Chart 1: IL18RAP is among the genes whose expression is most significantly different transcriptome-wide when comparing metastases to the lymph nodes and to the bone in human breast cancer.

The rank of global differential expression, the 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: 605
Probe ID: 207072_at
p-value: 5.55e-03
t: -2.883755
B: -2.26998
Gene: IL18RAP
Gene name: interleukin 18 receptor accessory protein

Chart 2: IL18RAP is among the genes whose expression is most significantly different transcriptome-wide when comparing metastases to the lymph nodes and to the bone in human breast cancer.

The rank of global differential expression, the 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: IL18RAP expression is significantly higher in bone metastasis when compared to lymph node metastasis.

The mRNA expression level of IL18RAP in metastasis to the lymph nodes (left) and in metastasis to the bones in women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in IL18RAP expression between metastasis to the lymph nodes and to the bones, a p -value, is $p=0.00005$.

Figure 2: IL18RAP primary tumor expression is correlated with overall survival in patients with breast cancer, in lymph node negative patients but not in lymph node positive patients.

Depicted in these Kaplan-Meier plots is the probability of overall survival in $n=452$ lymph node positive patients (**left**) and $n=726$ lymph node negative patients (**right**), based on low or high expression of IL18RAP in patient primary tumors, in the upper survival quartile. 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 IL18RAP 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.