NLRP12 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 data¹, comparing global gene expression profiles of metastasis to the bones and to the lymph nodes. We discovered that the nucleotide-binding oligomerization domain leucine-rich region (NLR) pyrin domain containing family protein *NLRP12* was among the genes whose expression was most different, transcriptome-wide, in bone and lymph node metastases. *NLRP12* 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 *NLRP12* in primary tumors of the breast was correlated with recurrence-free survival, in lymph node positive patients but not in lymph node negative patients. *NLRP12* has functions in transcriptional regulation of immune gene expression^{2,3} and in pathogen recognition of *Yersinia pestis*⁴.

Keywords: breast cancer, metastasis, bone metastasis, lymph node metastasis, NLR family pyrin domain containing 12, *NLRP12*, 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 metastases¹. We discovered transcriptome-wide differential expression of the gene encoding NLR family pyrin domain containing 12, NLRP12, in the bone metastases of patients with metastatic breast cancer.

Methods

We used dataset GSE46141¹ 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. 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 NLRP12 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 tool⁵ for correlation of NLRP12 mRNA expression levels with recurrence-free survival in $n=814$ lymph node positive patients and $n=574$ 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.

NLRP12 is differentially expressed in bone metastasis in human breast cancer.

Through blind, systems-level analysis of published microarray data¹, we identified NLR family pyrin domain containing 12, encoded by NLRP12 (Monarch-1), 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, NLRP12 ranked 82 out of 51565 total transcripts (Chart 1), equating to 99.8% differential expression. Differential expression of NLRP12 in the bone metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.55e-04$).

NLRP12 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for NLRP12, in bone and lymph node metastatic tissues in patients with metastatic breast cancer to determine direction and statistical significance of change in NLRP12 expression between metastasis to disparate sites. We observed significantly increased expression of NLRP12 in bone metastases of patients with breast cancer as compared to lymph node metastases: NLRP12 was expressed at 5.72 ± 1.19 arbitrary units (AU) in lymph node metastases, while it was expressed at 8.06 ± 1.42 AU in bone metastatic tissues (Figure 1). The difference in NLRP12 mRNA levels between metastasis to the bones and to the lymph nodes was statistically significant (Figure 1; $p=0.00022$).

NLRP12 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁵ in $n=814$ lymph node positive breast cancer patients and $n=574$ lymph node negative breast cancer patients, to evaluate whether NLRP12 tumor expression was correlated with survival outcomes in breast cancer and to evaluate whether lymph node status influenced correlation of NLRP12 tumor expression with survival outcomes in human breast cancer, in the lower survival quartile. We observed a correlation between primary tumor expression of NLRP12 and recurrence-free survival (RFS) in lymph node positive breast cancer (Figure 2). Lymph node positive patients whose primary tumors expressed low levels of NLRP12 possessed, on average, superior median RFS as compared to lymph node positive patients whose tumors expressed high levels of NLRP12. This difference in RFS based on NLRP12 tumor expression in lymph node positive patients with breast cancer trended towards statistical significance (Figure 2, Chart 3; logrank p -value: 0.075; hazard ratio: 1.29 (0.97-1.72)). NLRP12 primary tumor expression was not correlated with recurrence-free survival outcomes in lymph node negative patients (Figure 2, Chart 3; logrank p -value: 0.96; hazard ratio: 1.01 (0.66-1.55)).

Thus, by mining published microarray data¹ in an unbiased and systematic fashion, we identified NLRP12 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 NLRP12 in bone metastases. Further, we observed correlation between NLRP12 expression and recurrence-free survival in lymph node positive patients but not in lymph node negative patients.

Discussion

We provided evidence here that a member of the NLR family, NLRP12 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. It is unclear what purpose up-regulation of NLRP12 in the bone might serve. NLRP12 expression has traditionally been thought to be restricted to immune cells, but literature describes expression of NLRP12 in the gastrointestinal tract, and in immune organs such as the spleen and lymph nodes^{6,7}. NLRP12 has a specific function in immune defense against infections with *Yersinia pestis*, the causative agent of the bubonic plague, which was ascribed to pathogen sensing of *Y. pestis* (pattern recognition)⁴. This conclusion was, in part, based on the demonstration that release of both IL-1 β and IL-18 in NLRP12^{-/-} mice is reduced in response to *Y. pestis* infection despite otherwise intact inflammasome signaling as evidenced by normal responses to poly (dA:dT), alum, and nigericin⁴. It has not yet been demonstrated what is/are the ligand(s) for NLRP12. NLRP12 is not required for defense against *Klebsiella pneumoniae* and *M. tuberculosis*⁸, and although NLRP12 mice are resistant to infection with *Salmonella enterica* serovar Typhimurium, this does not involve perturbation of the inflammasome³. Thus, the requirement for NLRP12 in immune defense through pattern recognition appears to be relatively specific to *Yersinia pestis*. NLRP12 has also been shown to regulate production of interleukin-4 in T-lymphocytes², and to influence the migration of dendritic cells and macrophages⁹. NLRP12, through its ability to regulate gene expression of ERK and NF- κ B in macrophages, has been reported to promote tumorigenesis in the colon⁷. Thus, NLRP12 has functions in both innate immunity and in cancer.

Though unbiased, systems-level comparative transcriptome analysis of metastasis to disparate sites in human breast cancer, we identified NLRP12 differential expression and up-regulation in metastasis to the bones. Future studies will reveal whether any other genes important for modulation of innate immune gene expression, or for pattern recognition and immune defense against *Yersinia* (or other pathogens) are similarly transcriptionally modulated in metastatic breast cancer in humans.

References

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Rank: 82
Probe ID: 100146361_TGI_at
p-value: 1.55e-04
t: -4.14
B: 0.77569
Gene: NLRP12
Gene name: NLR family pyrin domain containing 12

Chart 1: NLRP12 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: NLRP12 expression is significantly higher in bone metastasis when compared to lymph node metastasis.

The mRNA expression level of NLRP12 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 NLRP12 expression between metastasis to the lymph nodes and to the bones, a p -value, is $p=0.00022$.

Figure 2: NLRP12 primary tumor expression is correlated with recurrence-free survival in patients with breast cancer, in lymph node positive patients but not in lymph node negative patients.

Depicted in these Kaplan-Meier plots is the probability of recurrence-free survival in $n=814$ lymph node positive patients (**left**) and $n=574$ lymph node negative patients (**right**), based on low or high expression of NLRP12 in patient primary tumors, 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 NLRP12 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.