Mediterranean fever (PYRIN) is differentially expressed and up-regulated in the bone metastases of patients with metastatic breast cancer.

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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 pattern recognition receptor *PYRIN*, also known as Mediterranean fever (MEFV), was among the genes whose expression was most different, transcriptome-wide, in bone and lymph node metastases. *PYRIN* 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 *PYRIN* in primary tumors of the breast was correlated with distant metastasis-free survival, in lymph node positive patients but not in lymph node negative patients. *PYRIN* has functions in innate immune sensing of modifications of RhoGTP and the cytoskeleton by bacterial Type III secretion systems^{2,3} and is modulated by *Yersinia pestis*^{4,5}.

Keywords: breast cancer, metastasis, bone metastasis, lymph node metastasis, MEFV, *PYRIN*, Mediterranean fever, 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, PYRIN, 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 PYRIN 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 tool⁶ for correlation of PYRIN mRNA expression levels with distant metastasis-free survival in $n=889$ lymph node positive patients and $n=1309$ 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.

PYRIN 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 PYRIN (MEFV), 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, PYRIN ranked 11 out of 51565 total transcripts (Chart 1), equating to 99.98% differential expression. Differential expression of PYRIN in the bone metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=5.82e-06$).

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

We obtained exact mRNA expression levels for PYRIN, in bone and lymph node metastatic tissues in patients with metastatic breast cancer to determine direction and statistical significance of change in PYRIN expression between metastasis to disparate sites. We observed significantly increased expression of PYRIN in bone metastases of patients with breast cancer as compared to lymph node metastases: PYRIN was expressed at 5.12 ± 0.61 arbitrary units (AU) in lymph node metastases, while it was expressed at 6.64 ± 0.90 AU in bone metastatic tissues (Figure 1). The difference in PYRIN mRNA levels between metastasis to the bones and to the lymph nodes was statistically significant (Figure 1; $p=0.000013$).

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

We performed Kaplan-Meier survival analysis⁶ in $n=889$ lymph node positive breast cancer patients and $n=1309$ lymph node negative breast cancer patients, to evaluate whether PYRIN tumor expression was correlated with survival outcomes in breast cancer and to evaluate whether lymph node status influenced correlation of PYRIN tumor expression with survival outcomes in human breast cancer, in the lower survival quartile. We observed a correlation between primary tumor expression of PYRIN and distant metastasis-free survival (DMFS) in lymph node positive breast cancer, in the upper survival tertile (Figure 2). Lymph node positive patients whose primary tumors expressed low levels of PYRIN possessed median DMFS of 51.88 months as compared to a median DMFS of 28.75 months in lymph node positive patients whose tumors expressed high levels of PYRIN. This difference in DMFS based on PYRIN tumor expression in lymph node positive patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.013; hazard ratio: 1.4 (1.07-1.83)). PYRIN primary tumor expression was not correlated with distant metastasis-free survival outcomes in lymph node negative patients (Figure 2, Chart 3; logrank p -value: 0.74; hazard ratio: 0.95 (0.72-1.26)).

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

Discussion

We provided evidence here that a second pattern recognition receptor, PYRIN, 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. We have recently demonstrated that NLRP12 is similarly transcriptionally modulated in the bone metastasis of patients with breast cancer⁷. It is unclear what purpose up-regulation of PYRIN in the bone might serve. Pyrin serves to recognize modification of RhoGTP and the cytoskeleton induced by bacterial Type III secretion systems during bacterial infections: PYRIN senses glucosylation of Rho by TcdB of *Clostridium difficile*^{2,3}. PYRIN is antagonized by the *Yersinia* effector YopM during the course of *Yersinia pestis* infection, as YopM blocks IL-1 and IL-18 production induced by *Yersinia* YopE⁵. Importantly, mutations in the gene that encodes PYRIN, MEFV, have recently been shown to provide resistance against *Yersinia* infections in humans⁸.

Though unbiased, systems-level comparative transcriptome analysis of metastasis to disparate sites in human breast cancer, we identified PYRIN differential expression and up-regulation in metastasis to the bones. We have now demonstrated that two separate pattern recognition receptors required for defense against *Yersinia pestis* infection, or modulated during the course of *Yersinia pestis* infection, 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. These data pose the question: why do we find evidence of a *Yersinia pestis* infection fingerprint in human breast cancer?

References

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Rank: 11
Probe ID: 100303641_TGI_at
p-value: 5.82e-06
t: -5.15
B: 3.52302
Gene: MEFV
Gene name: Mediterranean fever

Chart 1: MEFV (PYRIN) 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: PYRIN expression is significantly higher in bone metastasis when compared to lymph node metastasis.

The mRNA expression level of PYRIN 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 PYRIN expression between metastasis to the lymph nodes and to the bones, a p -value, is $p=0.000013$.

Figure 2: PYRIN primary tumor expression is correlated with distant metastasis-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 distant metastasis-free survival in $n=889$ lymph node positive patients (**left**) and $n=1309$ lymph node negative patients (**right**), based on low or high expression of PYRIN in patient primary tumors, in the upper survival tertile. The log rank p -value denoting statistical significance of difference in distant metastasis-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 PYRIN 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.

Lymph node positive patients

Low PYRIN expression: 51.88 months

High PYRIN expression: 28.75 months

Chart 3: Median distant metastasis-free survival is inferior in lymph node positive breast cancer patients with high primary tumor expression of PYRIN.

The median DMFS (distant metastasis-free survival) of patients with low primary tumor expression of PYRIN and high primary tumor expression of PYRIN is listed in this chart, for $n=889$ lymph node positive breast cancer patients, in the upper survival tertile.