NPRL2: A New Target in Breast Cancer Treatment

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Background

It has been well established that genomic instability and mutation is a major hallmark of cancer.[1] However, due to deficiencies in mismatch repair, most breast tumors do not display high rates of mutational burdens compared to other cancers, suggesting there are alternative pathways better for evaluating risk of tumor progression.[2,3] Notably, studies from our team and others have revealed that cytosol DNA fragmentation activates DNA sensing pathways which in turn activate the innate immune response.[4,5] Consequently, our group decided to investigate molecular determinants of the c-GAS/STING pathways to see if defects in the S-DDR pathway may reveal biomarkers responsible for the development of intermediate breast tumors. After running a genetic screen, we selected NPRL2 as a top candidate in regulating the S-DDR.

NPRL2 is a primary component of the GATOR1 complex which has been linked to tumor suppression through its inhibitory interaction with mTORC1.[6] Although the definitive function of NPRL2 and its role in regulating the S-DDR is unknown, our preliminary studies showed interesting results.

Methods

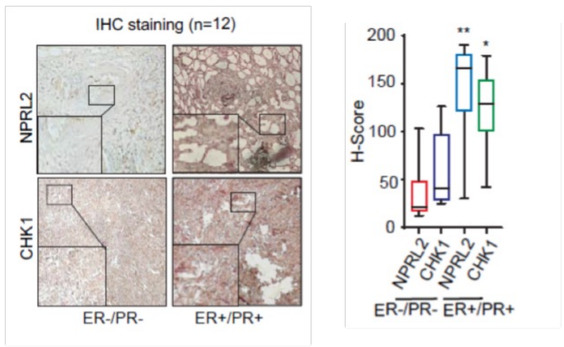
(1) **Immunohistochemistry (IHC)** to compare molecular differences in tissue microarray slides with breast lesions at various stages of breast cancer.
(2) **Bioinformatic Analysis** for breast cancer samples in TCGA
(3) **Generate NPRL2 knockdown cell lines using lentivirus**
(4) **q-PCR** (to see if NPRL2-/- or defects in the S-DDR promote innate immune signaling)

Expression of the following markers were analyzed:

- STING pathway factors: c-GAS, STING, CCL5, CXCL10, IFN-beta PD-L1, CD276

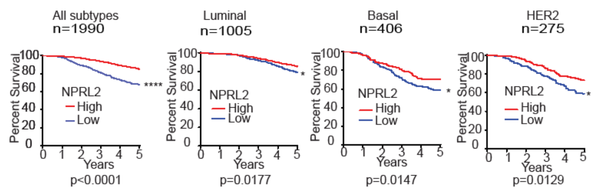
Results

Positive correlation between NPRL2 and CHK1 expression with Triple Negative Breast Cancer



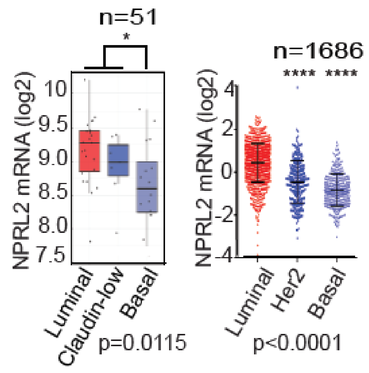
In breast cancer patient samples, we found reduced NPRL2 expression in triple negative breast cancer (TNBC) compared to hormone positive breast cancer. We also observed a positive correlation between NPRL2 expression and CHK1 expression, a key DNA damage kinase regulating genome stability. This data suggests that NPRL2 may play a previously unknown role in maintaining proper DNA damage response and genome integrity in TNBC by regulating key players in DDR (DNA damage response.)

Reduced NPRL2 expression in breast cancer patients is associated with a worse prognosis

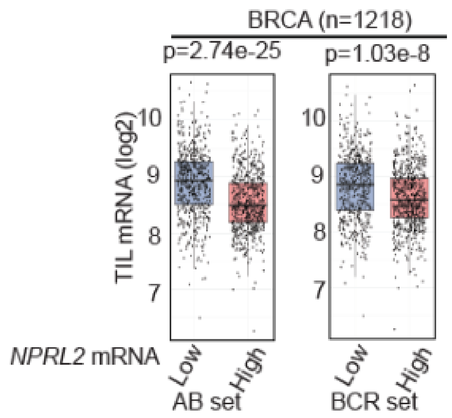


In breast cancer patient TCGA data (right), we found that reduced NPRL2 expression in breast tumors is associated with a worse prognosis in breast cancer patients. This association is not dependent on the breast subtypes. This data suggests that NPRL2 may play a role in breast tumor biology regardless of breast cancer types. NPRL2 deficiency could serve as a common target crossing different breast subtypes.

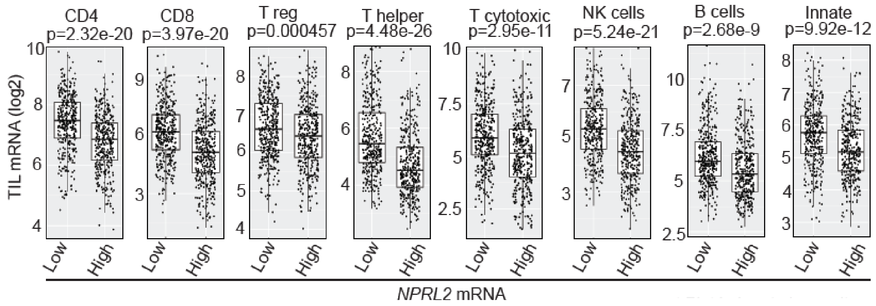
Reduced NPRL2 expression is associated with an increase in immune infiltration



In breast cancer cell lines (left) and patient TCGA data (right), we found reduced NPRL2 expression in basal like breast cancer (TNBC) compared to hormone receptor/HER2 positive breast cancer.

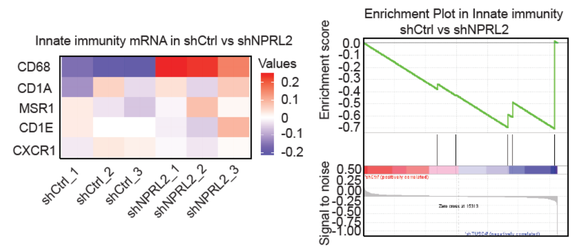


In breast cancer patient TCGA data, we found that reduced NPRL2 expression in breast tumors is associated with an increase immune infiltration, indicating a 'hot' immune status of tumors.



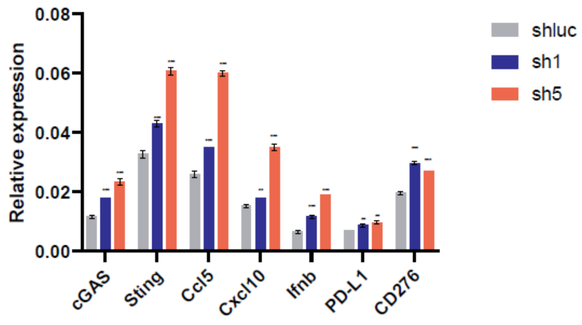
In breast cancer patient TCGA data, we found that reduced NPRL2 expression in breast tumors is associated with an increase infiltration of a variety of immune cells. This data further supports that NPRL2 deficiency may predict immune responses in breast tumors.

RNA-Seq Expression Analysis



In breast cancer cell lines, NPRL2 knockdown induces innate immune response analyzed by RNA-seq analysis, suggesting a DNA damage-dependent immune response might be active due to NPRL2 deficiency.

NPRL2 knockdown



NPRL2 knockdown induces c-GAS, STING, CCL5, CXCL10, IFN-beta, PD-L1, CD276.

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Conclusions

- **NPRL2 and CHK1 was positively correlated with TNBC**
- **Expression of NPRL2 was negatively correlated with prognosis of breast cancer patients**
- **Reduced NPRL2 expression in breast cancer induced innate immunity**

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