The mTOR Pathway Independent Function of NPRL2 in the Regulation of S-Phase DNA Damage Response

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Abstract:

We conducted a genetic screen to systematically identify molecular determinants of S-phase DNA damage response. We identified that NPRL2, a subunit of the GATOR1 complex, regulates S-DDR in an mTOR-pathway-independent fashion. Loss of NPRL2 reduces CHK1 protein stability and leads to defective S-DDR. NPRL2 deficiency enhances anti-tumor immunity through activation of DNA sensor STING-mediated innate immune response. We identify that NPRL2 is a key regulator of genome maintenance and anti-tumor immunity.

Introduction:

- The GATOR1 complex functions as a negative regulator of the mammalian target of rapamycin complex 1 (mTORC1) by activating RagA GTPase
- NPRL2 was a key mediator of sensitivity to irinotecan treatment, a topoisomerase I inhibitor, which suggests a possible role in S-phase DNA damage response.
- Mismatch repair (MMR) deficiency activates adaptive immune response and predicts responses of tumors to immune checkpoint blockade agents.
- NPRL2 expression was significantly lower in basal-like breast cancer
- Reduced mRNA expression of NPRL2 correlated strongly with poor cancer patient survival
- In melanoma and renal carcinoma studies, patients with low-NPRL2 expressing tumors showered better response to immune checkpoint blockade (PD-1/PD-L1)

Methods/Techniques:

Genomic Screening of S-Phase defective genes. 10169 genes were used to generate irinotecan sensitivity gene signatures after in-house normalization to average negative controls of each plate, comparison between average of high and average of medium/low cells lines were conducted to generate genomic similarity with Pan-Cancer Irinotecan sensitivity marker SLFN11.

Cell Culture. The breast and ovarian cancer cell lines were kindly provided by Dr. Gordon B. Mills' laboratory. The 293T and HS578T cells were maintained in DMEM. The other cell lines were maintained in RPMI1640 medium.

Multiplexed IHC staining. Tumor tissue from 4T1 underwent multiplexed immunofluorescence following the manufacturer's instruction (PerkinElmer). The following antibodies were used for IHC: anti-mouse PD-L1, anti-CD8, anti-STING, and anti-phospho-IRF3.

TIL scoring analysis and mutation load analysis. We analyzed the correlation of NPRL2 mRNA level and the immune signature scores and conducted FDR-based multiple testing correcting,

In Vivo *Mouse Models.* 4T1 breast cancer cells were injected into the mammary pads of 6-8 weeks old female mice per group. Tumor progression was monitored once per week.

Results :

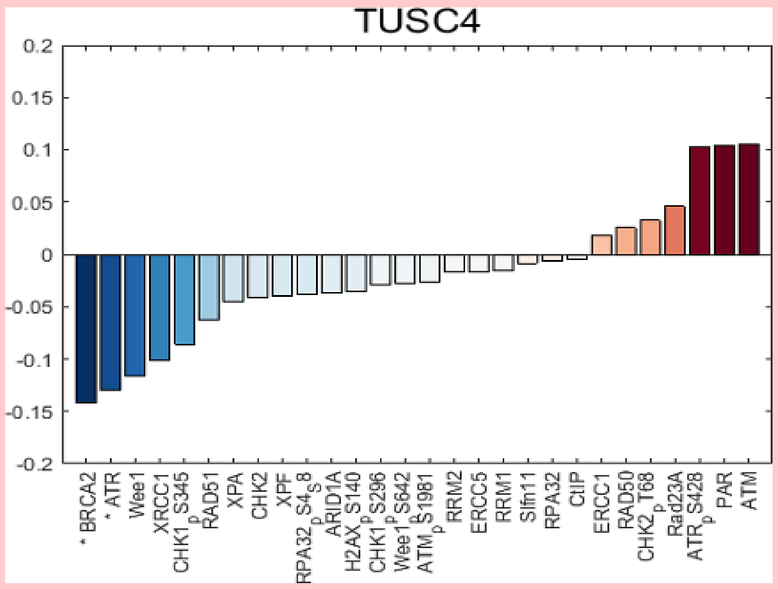

Figure 1. RPPA data analysis of KIRC TCGA data. DNA damage response and repair proteins that are associated with NPRL2 (also known as TUSC4) protein expression.

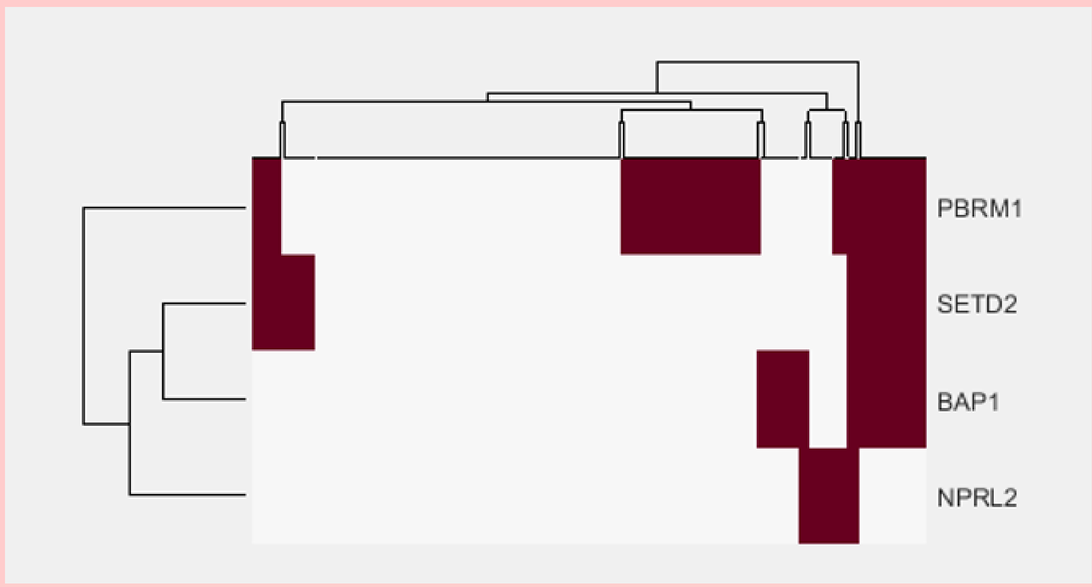

Figure 2. KIRC TCGA genomic data analysis of NPRL2/PBRM1/SETD2/BAP1-deleted/mutated/deficient tumors. The comparison of NPRL2 deficiency with additional key tumor suppressor in KIRC

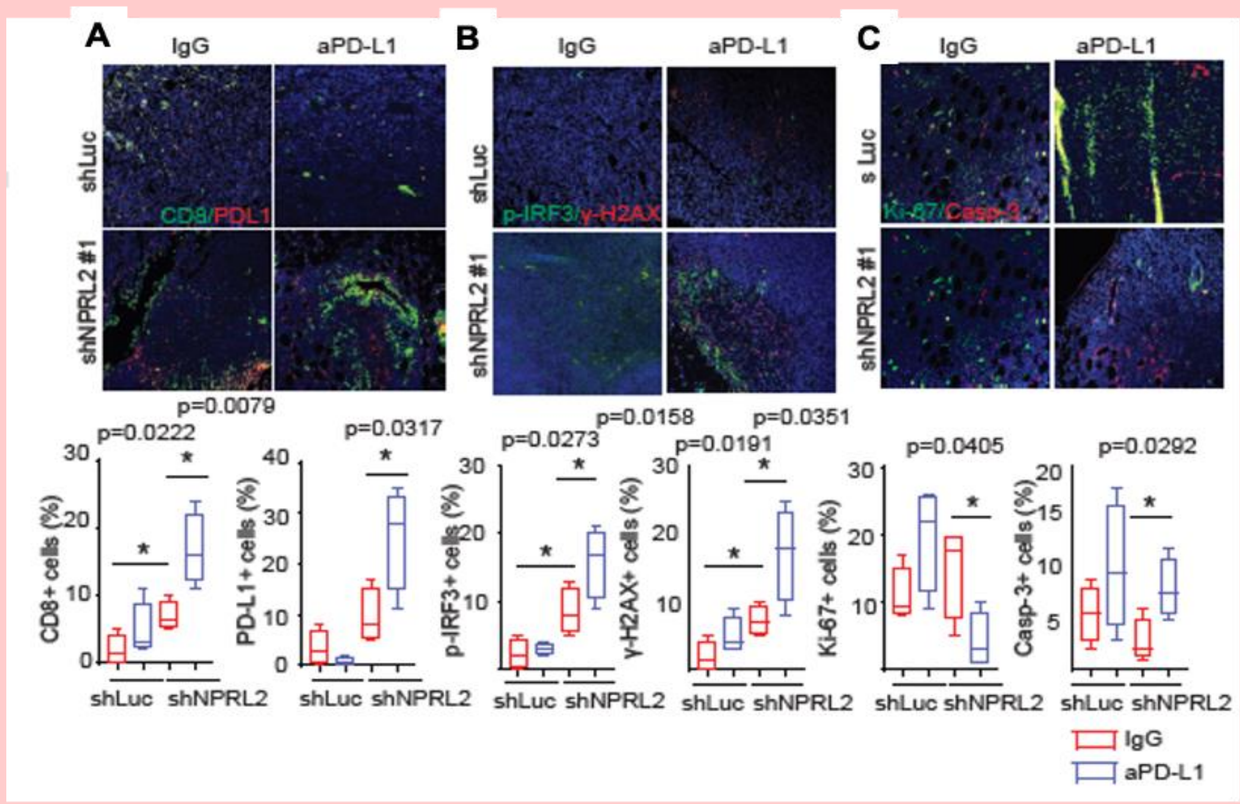

Figure 3. resentative multiplex-staining images from NPRL2-deficient mouse breast tumors. Representative images (Top) and quantitative analysis (Bottom) of CD8 and PD-L1 **(A)**, p-IRF3 and γ-H2AX **(B)**, and Ki-67 and Caspase-3 **(C)** in control and NPRL2-deficient tumors with indicated treatments. Data represent mean ± s.e.m. of three independent qPCR experiments; two-tailed *t* test. *p<0.05.

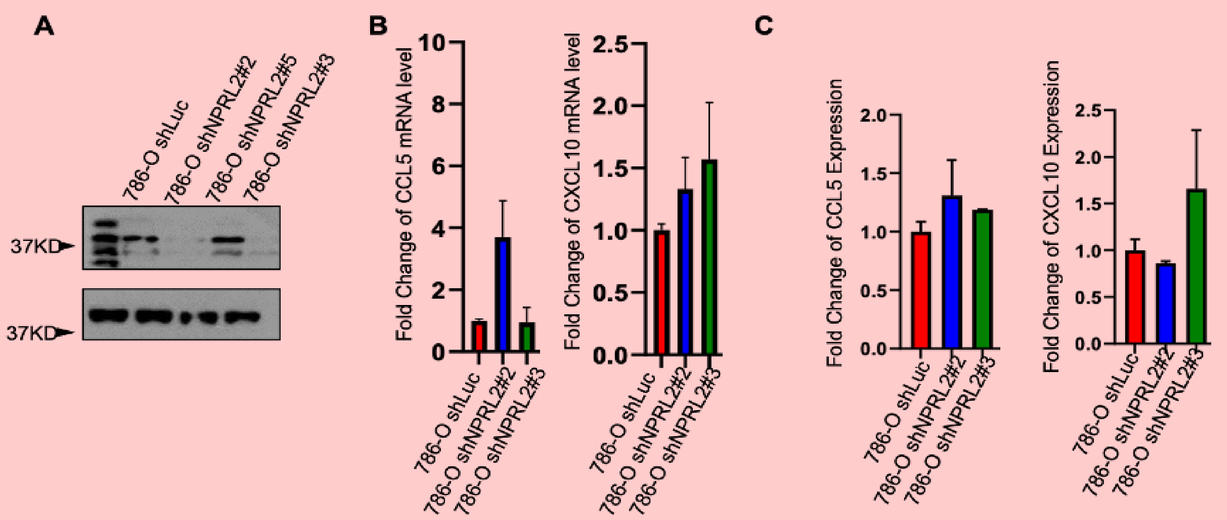

Figure 4. (A) The establishment of NPRL2 stable knockdown cells in ccRCC cancer cell lines. qPCR **(B)** and ELISA **(C)** analysis of CCL5 and CXCL10 in control and NPRL2-deficient tumors with indicated treatments. Data represent mean ± s.e.m. of three independent qPCR experiments; two-tailed *t* test. *p<0.05.

Discussion:

- The data suggests that the effect of NPRL2 on CHK1 protein stability is independent of its role in regulating mTOR signaling.
- DNA replication, recombination, and repair and cell cycle were among the top networks of proteins interacting with NPRL2, consistent with its role in S-DDR.
- Our findings demonstrate that NPRL2 deficiency promotes an innate immune response, suggesting that NPRL2 could determine immune responsiveness and may predict responses to immune checkpoint blockade in tumors with low mutation load.
- Results show that defective S-DDR as a tumor-intrinsic mechanism that activates the STING pathway and sensitizes tumors to anti-PD-L1 treatment.

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