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Targeting NCOR2 in tumor cells unleashes endogenous dsRNA sensing and enhances PD-1 blockade efficacy

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Short Report

Keywords:

Posted Date: November 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11697784/v1

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Additional Declarations: Competing interest reported. The corresponding author Xuetao Cao is the Editor-in-Chief of the journal Immunity & Inflammation. However, he is not involved in the peer-review or decision-making process for this manuscript. The authors declare no other competing interests.

Abstract

Transcriptional silencing of endogenous retroelements restricts the generation of immunostimulatory RNA to facilitate tumor immune evasion. Here, we identify NCOR2 as a tumor-intrinsic regulator of endogenous immunostimulatory RNA accumulation to suppress innate immune signaling. Tumor-cell NCOR2 knockdown suppresses 4T1 tumor growth and improves responses to PD-1 blockade, supporting further evaluation of NCOR2 as a target to enhance antitumor immunity.

Full Text

Tumor immune evasion is sustained in part by tumor cell-intrinsic mechanisms that limit the generation and recognition of immunogenic nucleic acids[1]. Transcription of endogenous repetitive elements can generate double-stranded RNA (dsRNA) that activates antiviral signaling and type I interferon responses[3, 4]. Epigenetic dysregulation is supposed to derepress endogenous repetitive elements to generate immunostimulatory RNA, but tumor cells can maintain innate immune silencing, suggesting active control of endogenous immunostimulatory RNA[2]. Epigenetic therapies that relieve transcriptional silencing can induce viral mimicry responses, highlighting transcriptional repression as a barrier to tumor immune recognition[3-5]. However, the specific corepressors that maintain this barrier and their regulation in tumor cells remain incompletely understood. Nuclear receptor corepressor 2 (NCOR2), also known as SMRT, mediates transcriptional repression through histone deacetylase-containing complexes[6]. Although NCOR2 has been implicated in breast cancer treatment resistance and interferon regulation, its role in controlling endogenous immunogenic RNA remains unclear[7, 8]. Here, we investigated whether NCOR2-mediated transcriptional repression restricts dsRNA accumulation and innate immune activation in tumor cells, and consequently impairs tumor immune recognition.

To unbiasedly identify tumor-intrinsic suppressors of immunogenic dsRNA accumulation, we performed a genome-wide CRISPR-Cas9 knockout screen in MDA-MB-231 human breast cancer cells. J2-stained dsRNA-high cells were sorted, followed by sgRNA sequencing and MAGeCK analysis. NCOR2 emerged as a candidate suppressor of endogenous dsRNA accumulation. Secondary siRNA screening of 21 candidate genes using J2 dot blot analysis further supported NCOR2 as a negative regulator of dsRNA abundance. These findings inspired us to investigate the role of NCOR2-mediated transcriptional repression in controlling endogenous immunogenic RNA generation and tumor-intrinsic innate immune activation.

We next examined the effects of NCOR2 depletion on tumor cell-intrinsic immune activation. RNA sequencing of si*NCOR2*-treated MCF7 human breast cancer cells revealed an antiviral transcriptional response, with enrichment of biological processes related to type I interferon responses and antiviral defense, accompanied by increased expression of interferon-responsive genes **(Fig. 1a, b; Supplementary Figs. 1 and 2)**. RT-qPCR in MDA-MB-231 cells confirmed increased mRNA levels of *IFIT1*, *OASL*, *STAT1*, *DDX58* (encoding RIG-I) and *IFIH1* (encoding MDA5) following *NCOR2* knockdown **(Fig. 1c)**.

NCOR2 depletion also increased endogenous dsRNA levels, as assessed by J2 dot blot, and elevated retroelement transcript abundance, including *Alu, HERV-L, HERV-K* and *LINE-1* **(Fig. 1d, e)**. Immunoblotting in MDA-MB-231 cells further showed increased RIG-I and mitochondrial antiviral signaling protein (MAVS) protein levels and TBK1 phosphorylation after *NCOR2* knockdown, consistent with activation of innate immune signaling **(Fig. 1f)**. Flow cytometry demonstrated that *NCOR2* silencing increased surface HLA class I expression in both MDA-MB-231 and MCF7 cells, which was reversed by concomitant silencing of MAVS, suggesting that MAVS-dependent signaling contributes to HLA class I upregulation following *NCOR2* depletion (**Fig. 1g**). Together, these results support a role for NCOR2 in restraining endogenous dsRNA accumulation and innate immune activation while limiting HLA class I expression in tumor cells.

We went further to examine the effects of tumor-cell *NCOR2* depletion on tumor growth and the response to PD-1 blockade *in vivo*. 4T1 murine breast cancer cells expressing shNCOR2 or a nontargeting control shRNA (shNC) were orthotopically implanted into the mammary fat pad, and tumors were harvested 25 days after inoculation. Compared with shNC controls, NCOR2-depleted tumors exhibited reduced growth, accompanied by an increased proportion of intratumoral $CD8^+$ T cells **(Fig. 1h, i)**. Anti-PD-1 treatment alone showed limited antitumor activity. However, combining PD-1 blockade with *NCOR2* knockdown more potently reduced tumor growth and increased the proportion of intratumoral $CD8^+$ T cells compared with *NCOR2* knockdown alone **(Fig. 1h, i)**. Therefore, *NCOR2* depletion suppresses tumor growth and enhances the efficacy of PD-1 blockade *in vivo*, accompanied by increased intratumoral proportion of $CD8^+$ T cells.

NCOR2 is reported in the epigenetic regulation of transposable elements in mouse embryonic stem cells[9]. To examine whether NCOR2 associates with retroelement-containing chromatin in tumor cells, we performed ChIP-qPCR in MDA-MB-231 cells. *Alu, HERV-L, HERV-K* and *LINE-1* sequences were significantly enriched in NCOR2 immunoprecipitates compared with IgG controls **(Fig. 1j).** Together with the increased abundance of these transcripts following *NCOR2* depletion, these findings support a chromatin-associated repressive role for NCOR2 in regulating retroelement expression in tumor cells.

Given the immunosuppressive role of TGF-β in the tumor microenvironment[10], we next examined whether TGF-β regulates NCOR2 expression. Treatment of MDA-MB-231 cells with recombinant TGF-β1 increased NCOR2 expression at both the mRNA and protein levels **(Fig. 1k, l)**. Increased SERPINE1 expression and SMAD2/3 phosphorylation confirmed activation of TGF-β signaling **(Supplementary Fig. 3a, b)**. These findings identify TGF-β1 as an upstream inducer of NCOR2 expression and suggest that microenvironmental TGF-β signaling may reinforce NCOR2-mediated suppression of tumor-intrinsic innate immunity.

To assess the clinical relevance of NCOR2 in cancer, we performed an exploratory KM Plotter analysis[11] in a receptor-defined triple-negative breast cancer (TNBC) subset. A directionally consistent association between high NCOR2 mRNA expression and shorter relapse-free survival (n = 392; HR = 1.52; log-rank P = 0.0498) was observed **(Supplementary Fig. 4a)**. In pooled pan-cancer cohorts

receiving anti-PD-1 therapy[11], high NCOR2 expression was associated with shorter overall survival (n = 411; HR = 1.68; log-rank P = 1.6×10^{-4}) and progression-free survival (n = 283; HR = 1.91; log-rank P = 7×10^{-5}) **(Fig. 1m; Supplementary Fig. 4b)**. These observations connect higher NCOR2 expression with adverse clinical outcomes, including among patients receiving anti-PD-1 therapy.

Our findings support a role for NCOR2 in tumor-intrinsic control of immunostimulatory RNA. Previous studies implicated NCOR2 in transposable element regulation in embryonic stem cells[9] and in breast cancer immune evasion through HDAC3-associated repression of IRF1-regulated genes and MHC class I antigen presentation[7, 8]. Our data suggest that restriction of endogenous immunostimulatory RNA accumulation may also contribute to NCOR2-associated immune evasion, supporting tumor transcriptional dysregulation as a safeguard against immune recognition. TGF-β1-induced NCOR2 expression further suggests a potential connection between tumor microenvironmental signals and intrinsic safeguards. Several questions still remain. ChIP-qPCR data confirm NCOR2 occupancy at the assayed retroelement regions but do not establish the chromatin-based mechanism underlying transcriptional repression. Functional studies are also needed to determine whether NCOR2 mediates the immunosuppressive effects of TGF-β. Despite these limitations, the improved response to PD-1 blockade supports further evaluation of tumor-cell NCOR2 intervention as a strategy to enhance antitumor immunity.

Declarations

Data availability

The RNA-seq data generated from NCOR2-silenced and control cells in this study have been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE352437.

Fundings

This work was supported by grants from the National Natural Science Foundation of China (82388201) and Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (2025-I2M-XHZY-019, 2024-I2M-2-001-1, 2024-PT310-01) and UK Medical Research Council (MR/Y015347/1).

Contributions

X.C. designed the experimental approach and supervised the study; P.Z., L.L. H.L., and H.S. performed CRISPR screening, flow cytometry analysis, RT-qPCR and immunoblotting; T.D. provided helpful discussion and suggestions of data interpretation. P.Z., L.L., B.C. and X.C. analyzed data and wrote the paper.

Ethics declarations

Ethics approval and consent to participate

All experimental procedures involving animal experiments in this study were approved by the Animal Care and Use Committees of the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences under assurance identification number ACUC-XMSB-2024-205.

Consent for publication

Not applicable.

Competing interests

The corresponding author Xuetao Cao is the Editor-in-Chief of the journal Immunity & Inflammation. However, he was not involved in the peer-review or decision-making process for this manuscript. The authors declare no other competing interests.

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Figures

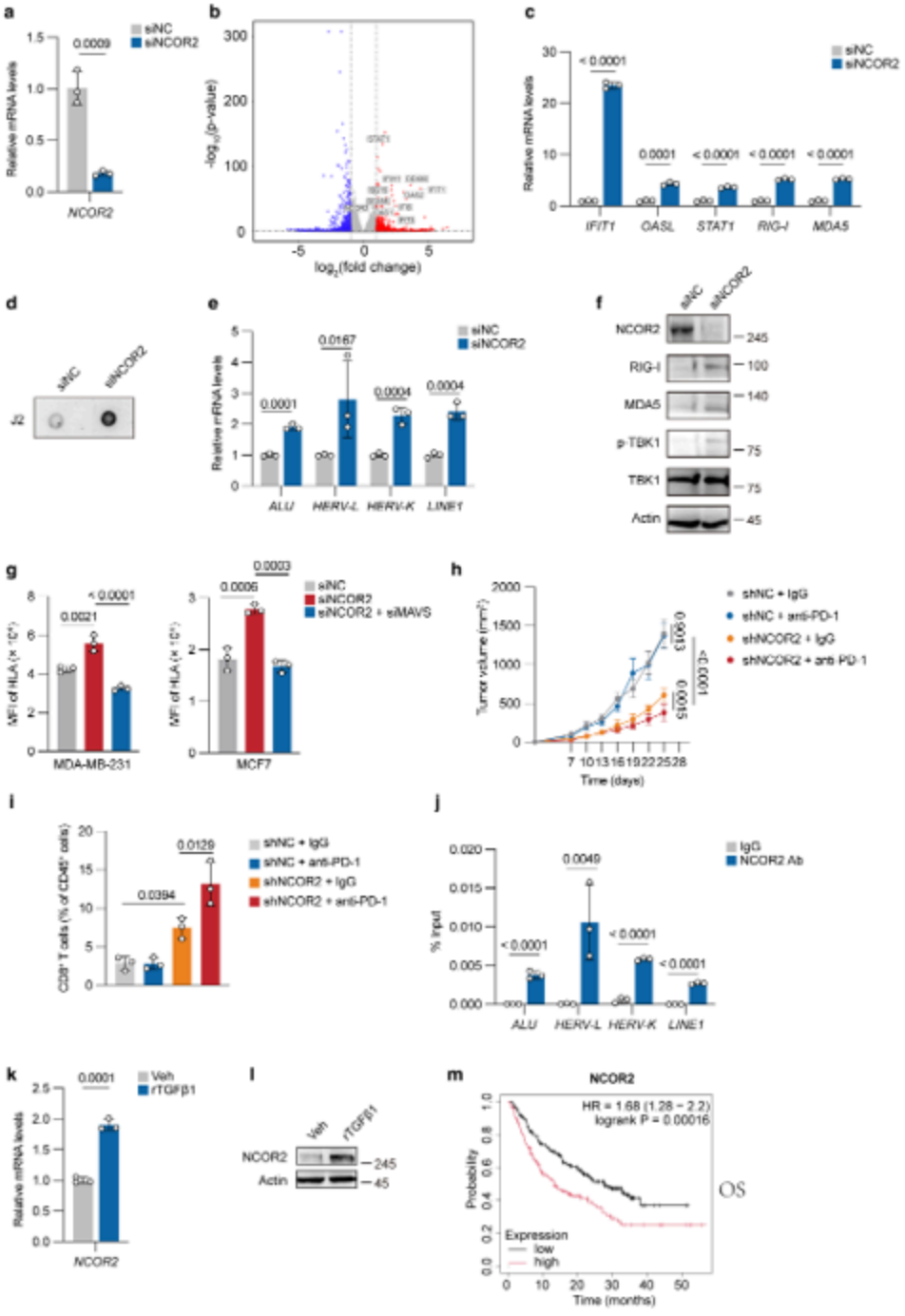

Figure 1

Tumor cell-intrinsic NCOR2 suppresses endogenous dsRNA-driven innate immune activation to limit antitumor immunity. a, RT-qPCR analysis of *NCOR2* mRNA in MCF7 cells transfected with *NCOR2*-targeting siRNA (si*NCOR2*) or nontargeting control siRNA (siNC). **b,** Volcano plot showing differential gene expression identified by RNA-seq in si*NCOR2*-transfected MCF7 cells compared with siNC controls. Red and blue indicate upregulated and downregulated genes, respectively; selected genes are labeled. **c,**RT-qPCR analysis of IFIT1, OASL, STAT1, DDX58 (encoding RIG-I) and IFIH1 (encoding MDA5) mRNA in MDA-MB-231 cells following *NCOR2* knockdown. **d, e,** J2 dot blot analysis of dsRNA **(d)** and RT-qPCR

analysis of Alu, HERV-L, HERV-K and LINE-1 transcripts **(e)** in MDA-MB-231 cells transfected with siNC or si*NCOR2*. **f,** Immunoblot analysis of NCOR2, RIG-I, MDA5, phosphorylated TBK1 (p-TBK1) and total TBK1 in MDA-MB-231 cells following NCOR2 knockdown. **g,** Flow cytometric analysis of surface HLA class I expression in MDA-MB-231 and MCF7 cells transfected with siNC, si*NCOR2*or si*NCOR2* together with *MAVS*-targeting siRNA (si*MAVS*). **h, i,** Tumor growth curves **(h)** and frequencies of intratumoral $CD8^+$ T cells among $CD45^+$ cells **(i)** in BALB/c mice bearing orthotopic 4T1 tumors expressing control shRNA (shNC) or NCOR2-targeting shRNA (shNCOR2), treated with anti-PD-1 antibody or isotype control IgG (200 μg per mouse per dose, intraperitoneally) on days 10, 13 and 16 after tumor inoculation. **j,**ChIP-qPCR analysis of NCOR2 association with Alu, HERV-L, HERV-K and LINE-1 sequences in MDA-MB-231 cells using an anti-NCOR2 antibody or IgG control. Enrichment is expressed as a percentage of input. **k, l,** RT-qPCR **(k)**and immunoblot **(l)** analysis of NCOR2 expression in MDA-MB-231 cells treated with vehicle (Veh) or recombinant TGF-β1 (rTGF-β1; 5 ng/mL) for 36 h. Actin served as a loading control in **f** and **l**; molecular masses are indicated in kDa. **m,** Kaplan-Meier analysis of overall survival in pooled pan-cancer cohorts receiving anti-PD-1 therapy, stratified by NCOR2 mRNA expression (n = 411). The hazard ratio (HR) with its 95% confidence interval and log-rank P value are shown. Tick marks indicate censored observations. Data are representative of two or three independent experiments (**a, c-l**). Data are presented as mean ± s.d. Statistical significance was determined by two-sided unpaired *t*-tests (**a, c, e, j, k**), one-way ANOVA (**g, i**), two-way ANOVA (**h**), log-rank tests (**m**).

Supplementary Files

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