

JNK1 and JNK2 Promote Triple-Negative Breast Cancer Aggressiveness by Fostering an M2 Macrophage–Driven Immunosuppressive Tumor Microenvironment

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Introduction

- Triple-negative breast cancer (TNBC) is characterized by an immunologically cold tumor microenvironment (TME), which facilitates immune evasion and tumor progression.[1]
- TNBC's immunosuppressive TME limits therapeutic efficacy;[2] reprogramming TME to restore immune response could lead to better therapy response.
- JNK, a key kinase in the MAPK pathway, orchestrates inflammation and fibrosis, and promotes tumor progression,[3] and has been targeted in clinical trials for inflammatory diseases (NCT04048876) and solid tumors (NCT05625412).
- Our previous study showed that JNK signaling promotes an immunosuppressive TME in TNBC via macrophage–derived CCL2, driving tumor growth and metastasis.[4]
- JNK1 and JNK2 isoforms have been implicated in the development of various cancers,[5] but their distinct roles in regulating TME immune landscape and aggressiveness in TNBC remain unknown.

Objective & Hypothesis

- **Objective**: Define the distinct roles of JNK1 and JNK2 in regulating the TME immune landscape and aggressiveness in TNBC.
- **Hypothesis**: Both JNK1 and JNK2 promote TNBC aggressiveness by fostering an M2 macrophage–driven immunosuppressive TME.

Results

Inhibition of JNK signaling suppressed PyMT-M Cell Growth *in vitro*

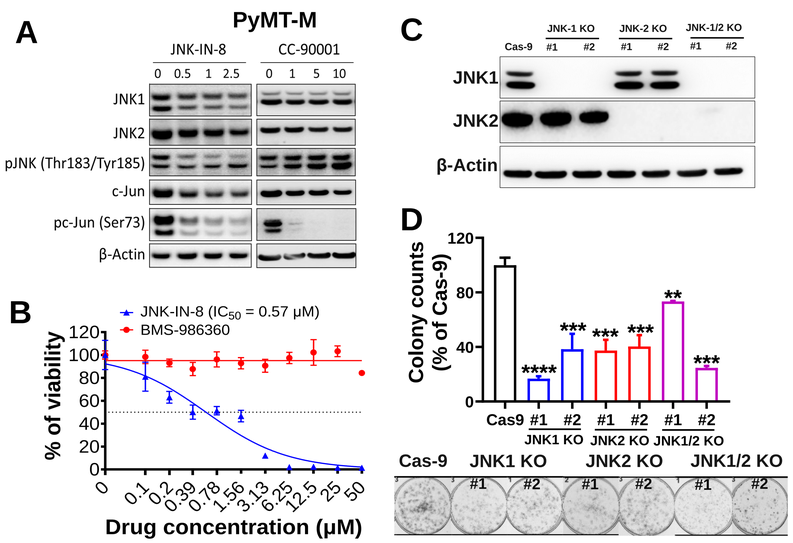


Figure 1. **A** and **B,** Inhibition of JNK signaling by JNK-IN-8 and CC-90001 (**A**) suppressed c-Jun phosphorylation, determined by Western blotting following 48-h treatment, and (**B**) inhibited cell proliferation, determined by CellTiter-Blue Cell Viability Assay following 72-h treatment in murine PyMT-M TNBC cells. **C** and **D,** (**C**) Knockout (KO) of JNK1, JNK2, or both using CRISPR-Cas9 suppressed PyMT-M cell growth, assessed using the clonogenic assay.

Intratumoral JNK KO Did Not Affect Tumor Growth and Immune Cell Tumor Infiltration *in vivo*

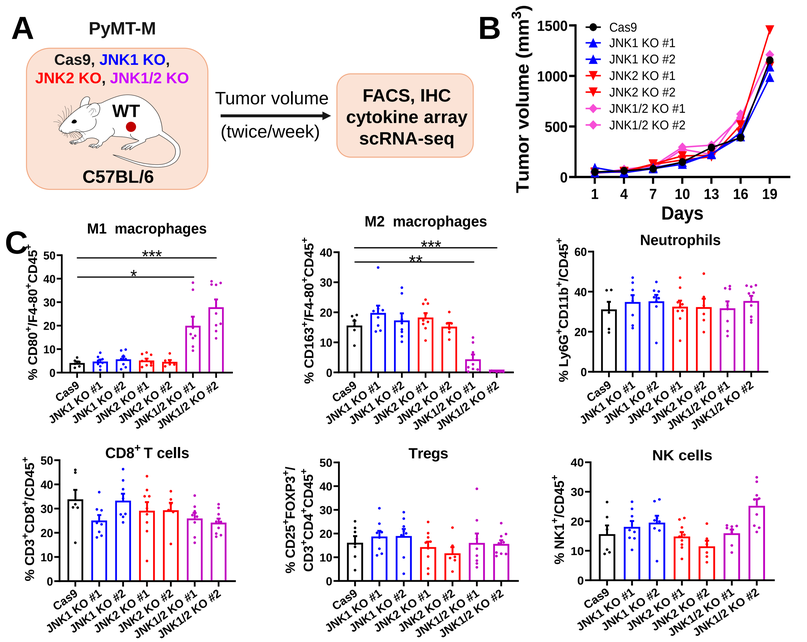


Figure 2. **A**, Schematic of the workflow. **B** and **C**, Compared to the Cas9 control, intratumoral KO of either JNK1, JNK2, or both in PyMT-M cells did not affect **(B)** tumor initiation and growth, or **(C)** immune cell tumor infiltration in the wild-type (WT) immunocompetent syngeneic C57BL/6 mice. * P < 0 .05; ** P < 0.01; *** P < 0.0001.

JNK Deficiency in the TME Delayed Tumor Initiation, Reduced Tumor Growth, and Induced Reprogramming of M2 to M1 Macrophages

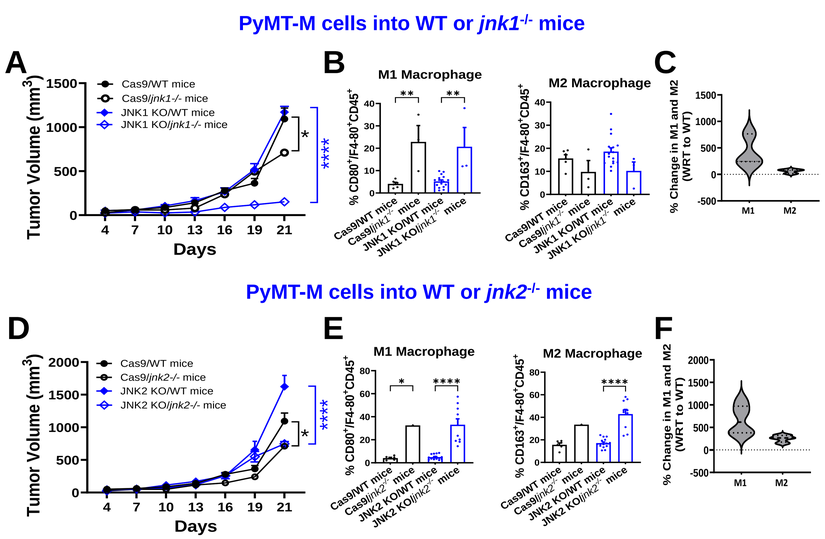


Figure 3. **A-F**, Regardless of the absence or presence of JNK1 and JNK2 in tumor cells, deficiency of either (**A-C**) *jnk1* or (**D-F**) *jnk2* in the TME (**A** and **D**) delayed tumor initiation and suppressed tumor growth, (**B** and **E**) increased M1 macrophage populations and reduced M2 macrophage populations, and (**C** and **F**) increased M1/M2 macrophage ratios. * P < 0 .05; ** P < 0.01; **** P < 0.0001.

Key Cytokines Involved in JNK-Promoted TME Immunosuppression and Tumor Growth

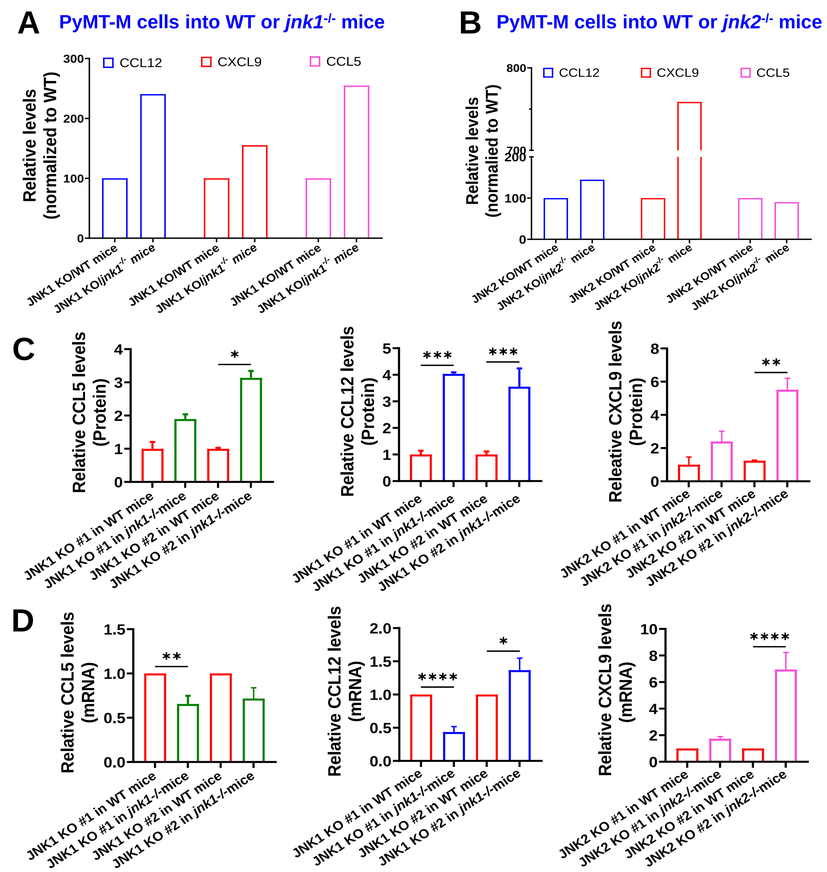


Figure 4. **A-D**, Compared to PyMT-M tumors from WT C57BL/6 mice, (**A**) expression levels of CCL12, CCL5, and CXCL9 were higher in JNK1 KO tumors from *jnk1*$^{-/-}$ C57BL/6 mice, and (**B**) those of CXCL9 were higher in JNK2 KO tumors from *jnk2*$^{-/-}$ C57BL/6 mice, determined by (**A** and **B**) cytokine array analyses and validated by (**C**) ELISA and (**D**) RT-PCR. * P < 0 .05; ** P < 0.01; *** P < 0.0001; **** P < 0.0001

Conclusion and future studies

- Intratumoral JNK1 and JNK2 are not required for PyMT-M tumor initiation and progression.
- JNK1 and JNK2 in the TME contribute to TNBC growth by promoting an M2 macrophage–driven immunosuppressive TME.
- JNK1 and JNK2 are potential therapeutic targets in TNBC.
- Investigate how JNK1 and JNK2 promote TME immunosuppression as well as TNBC initiation and progression via CCL12, CCL5, or CXCL9

Acknowledgements

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References

1. PMID: 39000314; 2. PMID: 36657483; 3. PMID: 32252279; 4. PMID: 34250544; 5. PMID: 34502556