Abstract #2498

Targeting Pro-Metastatic Kinase Networks in Triple-Negative Breast Cancer

Margarite D. Matossian[1], Madeline Bungert[1], Long Chi Nguyen[1], Michael East[3], Dennis Okumu[3], Rita Nanda[3], Gary Johnson[3], Marsha Rich Rosner[1]

[1]Ben May Department for Cancer Research, [2]Department of Medicine, The University of Chicago; [2]Department of Pharmacology, University of North Carolina

THE UNIVERSITY OF CHICAGO

Abstract

Background: Metastatic triple-negative breast cancer (mTNBC) is one of the most challenging forms of breast cancer to treat due to profound heterogeneity and drug resistance. There have been very limited advancements in approval of targeted treatment regimens that are not chemotherapy-based for mTNBC in the past decade. Kinases are tractable therapeutic targets, but while kinase inhibitors have improved outcomes in many metastatic solid tumors including other breast cancer subtypes, they have not improved patient outcomes in TNBC clinical trials. We posit this is because we do not leverage understanding of complex kinase signaling networks and how we utilize kinase inhibitors in TNBC is not optimized. Further, high doses of kinase inhibitors to achieve cytotoxicity have unacceptable side effects for patients limiting real-world use. Chronic stress states such as those initiated by hypoxia, poor nutrient delivery and higher metabolic demands promote pro-metastatic transcriptional programs in TNBC. Preclinical research and secondary analyses from clinical trial data reveal stress kinase signaling networks, and not individual pathways, drives TNBC metastatic transformation. Our central hypothesis is the RKIP/BACH1 signaling axis is a regulator of the chronic stress response to suppress TNBC metastasis and can be leveraged to produce biomarkers for risk stratification and a novel combination regimen.

Methods: Prior work showed activation of the physiologic metastasis suppressor RKIP effectively reduced TNBC metastasis in cell line models through inhibition of stress MAPK networks (p38, JNK, ERK1/2, MLK) resulting in decreased transcription of the pro-metastatic transcription factor BACH1. We utilized mass spectrometry-based techniques with a kinase inhibitor system (MIB-MS) to characterize the activity of kinase signaling networks in heavily treated mTNBC organoids and examined kinase networks prominent in BACH1-high TNBC from publicly available datasets. We utilize live/dead and proliferation assays and transwell invasion assays to test efficacy of our stress kinase inhibitor regimen.

Results: We show that combinations of 3 kinase inhibitors used in early phase clinical trials (Ralimetinib (p38), JNK-in-8 (JNK) or FDA approved (Trametinib (MEK & ERK1/2)) inhibit stress kinase networks, pro-motility gene expression and TNBC cell invasion. Low dose inhibition of multiple kinase nodes of networks, rather than single kinase nodes, more effectively reduces oncogenic signaling output and prevents compensatory signaling activation and potentiates suppression of organoid tumor initiating capacity when used in combination with chemotherapy. Using patient-derived organoids that recapitulate heterogeneous mTNBC molecular characteristics we successfully generated stable BACH1-knockout organoids which will be used to further identify BACH1-dependent critical kinase networks.

Conclusions: Inhibition of these stress kinase networks should enable effective reprogramming of metastatic or high-risk cancers to more benign states and make tumors more susceptible to approved anti-proliferative therapies such as chemotherapy or immunotherapy while limiting toxicities from kinase inhibitors using a low-dose, multi-drug combination approach.

Low-dose, multi-kinase inhibition approach

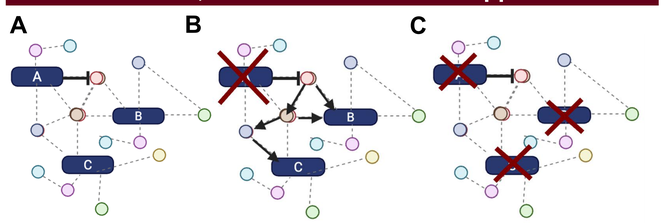


Fig. 1 | Treatment strategies to reduce stress oncogenic signaling output. A) Example of kinase network interactions including negative feedback between neighboring pathways. B) Schematic of how classic approaches to multi-kinase inhibition activate neighboring signaling pathways and contribute to kinase inhibitor resistance. C) Our novel approach to multikinase inhibition using lower doses and targeting multiple critical kinase nodes within networks to prevent compensatory signaling activation.

Acknowledgements

- We are incredibly grateful to the patients and their families who donated tissue for research
- Organoids were obtained and maintained by the University of Chicago Organoid and Primary Culture Resource due to a generous donation from Suzanne Zaconne

References

[1]Yesilkanal et al, *JBC* 297(4): 101128 (2021)
[2]Wolf et al, *npj Breast Cancer* **6**, 48 (2020)
[3]Yesilkanal et al, *eLife* **10**:e59696 (2021)

THE UNIVERSITY OF CHICAGO | DEPARTMENT OF MEDICINE Section of Hematology/Oncology

NIH National Institutes of Health

RKIP activates stress MAPK networks to suppress TNBC metastasis

- RKIP is a physiologic metastasis suppressor
- BACH1 is a transcription factor downstream of RKIP
- RKIP and BACH1 regulate TNBC stress responses and metastasis
- Prior data using magnetic inhibitor bead (MIB-MS) kinase activity assays revealed RKIP activates stress MAPK pathways[1]

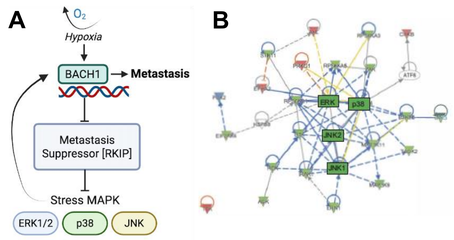


Fig. 2 | The RKIP/BACH1 axis functions through stress MAPK signaling. A) Kinase activity analysis from BM1 xenograft tumors with overexpressing RKIP compared to wildtype control. P38, JNK and ERK1/2 were kinase networks inhibited by RKIP. B) Schematic showing RKIP is a metastasis suppressor that inhibits stress MAPK networks that promote BACH1 activity. BACH1 then activates transcription that drives metastasis, immune evasion, glycolytic metabolism, drug resistance.

Signatures incorporating multiple stress kinase networks associated with worse response to neoadjuvant treatment in high-risk TNBC

- In a secondary analysis of the TNBC patient cohort of the adaptive ISPY-2 clinical trial, a stress phospho-kinase signature representing 3 kinase networks (MEK/ERK, p38, MSK1) correlated with worse response to neoadjuvant therapy in high-risk TNBC, but not in HER2+ tumors[2]
- TNBC biology is driven by multi-nodal MAPK and AKT stress signaling networks
- A signature representing 2 kinase networks (MEK/ERK, p38) was not significantly associated with neoadjuvant treatment response in TNBC

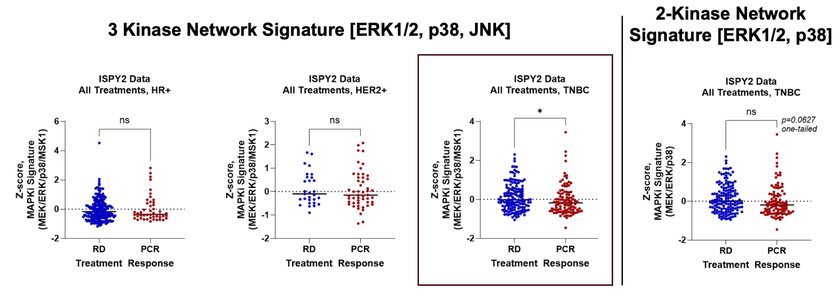


Fig. 3 | Stress phosphorylation signatures are associated with worse clinical outcomes in TNBC. A. Secondary analysis of ISPY-2 adaptive trial dataset that contains phosphorylated kinase array (RPPA-based) results using a surrogate stress kinase activation signature representing phosphorylated MAPK (MEK1/2, p38, MSK1) associated proteins and effectors. pCR = pathologic complete response. RD = residual disease.

Reduction of MAPK signaling through multi-kinase inhibition suppresses TNBC invasion and metastasis

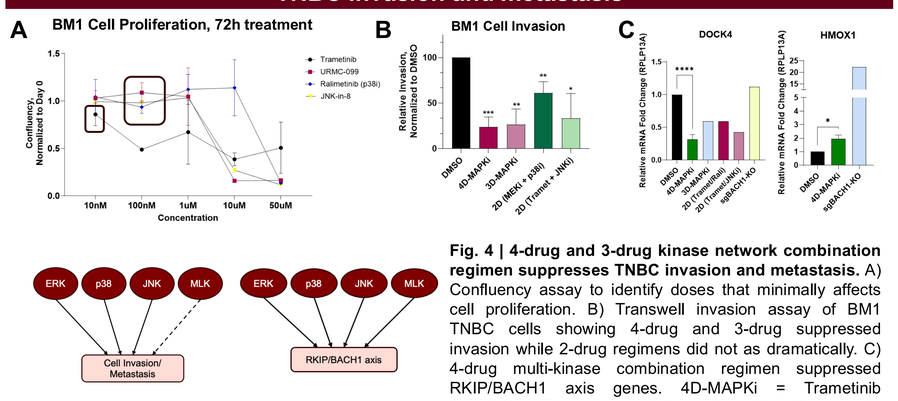


Fig. 4 | 4-drug and 3-drug kinase network combination regimen suppresses TNBC invasion and metastasis. A) Confluency assay to identify doses that minimally affects cell proliferation. B) Transwell invasion assay of BM1 TNBC cells showing 4-drug and 3-drug suppressed invasion while 2-drug regimens did not as dramatically. C) 4-drug multi-kinase combination regimen suppressed RKIP/BACH1 axis genes. 4D-MAPKi = Trametinib (MEK1/2i) + Ralimetinib (p38i) + JNK-in-8 (JNKi) + URMC-099 (MLKi); 3D-MAPKi = MEK1/2i + p38i + JNKi.

- Using a multi-kinase target inhibition approach with clinically relevant drugs mimics the action of RKIP, a physiologic metastasis suppressor, to suppress cell invasion/metastasis while suppressing BACH1 activity, similar to prior work[3]
- This strategy dissipates surplus signal, preventing threshold activation of compensatory kinase networks in TNBC.

Drug-resistant metastatic TNBC organoids

Organoid	Age	Site of origin	Grade	BC Subtype	Ki-67	Metastatic Sites Involved	TMB, MSS, PD-L1 Status	Genetics	Medical Tx
S021	56	Primary tumor	Grade 2	TNBC	15%	Bones, Skin	3.3 mut/MB MSS PD-L1+	TP53 c.743G>A, p.R248Q (VAF: 41%)	1L: carbo/pembro 2L: taxol 3L: Sacituzumab 4L: capecitabine
S034	57	Skin Met	Grade 3	TNBC /HER2-low	>75%	Skin	Unknown TMB, PD-L1-	Negative	1L: ddACT 2L: capecitabine 3L: taxol 4L: sacituzumab
H340	56	Breast (de novo met)	Grade 3	TNBC	60%	Liver, peritoneum, lungs, skin bones	N/A	Negative	1L: carbo/gem 2L: Saci + avelumab (trial)

- Organoids established in the Organoid and Primary Resource facility and established from patient biopsies, mostly from metastatic sites
- S021, S034, H340 represent drug resistant, metastatic TNBC tumors refractory to multiple lines of therapy
- All patients were exposed to a prior line of chemotherapy or an ADC

Low-dose MAPK inhibition prevents organoid formation

- The ability to form organoids is a property of self-renewing cells
- 3D-Stress MAPKi prevented organoid formation while 2D-MAPKi did not

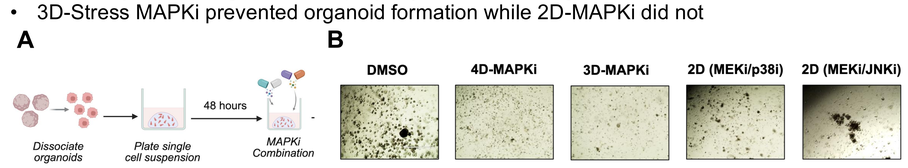


Fig. 5 | 3D-MAPKi and 4D-MAPKi prevented organoid formation. A) Schematic of organoid treatment. TNBC organoids were dissociated into single cells, plated in suspension for 48 hours, and treated with MAPKi combinoation. B) 4D-MAPKi and 3D-MAPKi combinations suppressed capability of organoid formation. 2D combinations did not have the same effect.

MAPK inhibition sensitizes TNBC cells and organoids to chemotherapy

- Taxane chemotherapy is used commonly in the first line setting for early-stage high risk and metastatic TNBC
- Inhibition with MEK1/2, p38 and JNK, or MEK1/2 and p38, most dramatically and consistentlys sensitized TNBC cells and organoids to taxane chemotherapy

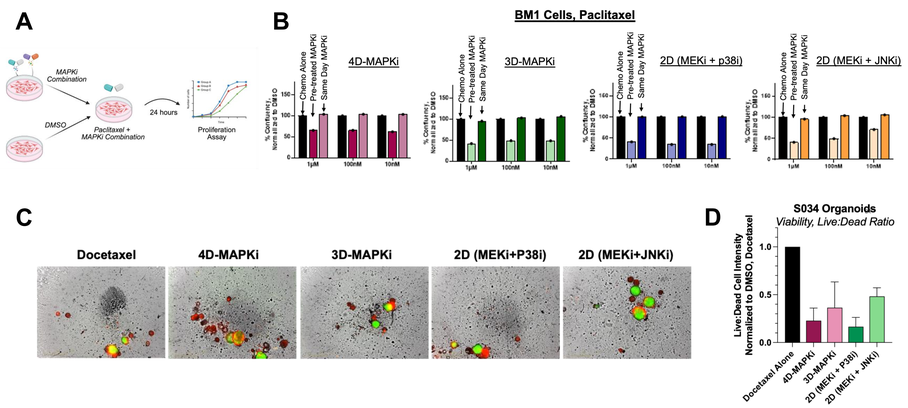


Fig. 6 | Stress MAPK inhibition sensitized cells and organoids to chemotherapy. A) Schematic of BM1 cell and S034 organoid treatment. TNBC organoids were treated intact with MAPKi or DMSO pre-treatment and then paclitaxel or docetaxol were added to the cells or organoids. B) 3D-MAPKi and MEKi/p38i combinations most dramatically sensitized BM1 cells to paclitaxel. C, D) 4D-MAPKi and MEKi/p38i most dramatically sensitized highly drug-resistant TNBC organoids to docetaxel.

Preliminary Conclusions & Future Directions

- TNBC metastasis is driven by stress phenotypes, and new strategies to target stress signaling networks are necessary to effectively manage metastasis.
- Inhibition of critical kinase signaling nodes at the center of networks instead of individual kinases is a novel kinase combination strategy
- Stress MAPKi regimen targeting 3 stress networks is effective in multiple TNBC models and suppresses invasion and organoid formation, and enhances sensitivity to chemotherapy
- Future studies are exploring how this combination reprograms kinase networks and metastatic transcription through proteomics and RNA sequencing
- Ongoing work using kinase network identification through kinase activity analyses has led to identification of novel mechanisms of resistance and development of therapies that can overcome therapeutic resistance