

A 07 Mena^{INV} a novel therapeutic target for prevention and treatment of metastatic disease

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Background

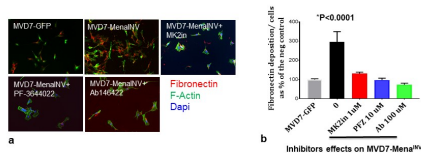
- Mena, an Ena/VASP protein family member, is a key actin regulatory protein and driver of metastasis
- Up-regulated in solid cancers and promotes tumor cell invasion, motility, and metastasis
- Mena^{INV} is a protein isoform of Mena and is significantly associated with cancer metastasis and decreased overall patient survival
- Paclitaxel promotes breast cancer dissemination in a Mena-dependent manner

Key Findings

Here we report inhibiting MAPKAPK2 kinase activity reverses Mena^{INV}-driven aggressive tumor cell phenotypes *in vitro*, and significantly reduces metastasis *in vivo* in a murine model of breast cancer. We identified a therapeutic target responsible for the activation of the Mena^{INV} pathway when inhibited can reverse paclitaxel-mediated pro-metastatic changes, resistance to paclitaxel and cancer cell dissemination in advanced cancer.

Fibronectin Fibrillogenesis Assay

Inhibition of MK2 reduced Mena-dependent fibrogenesis phenotype to Mena-null levels

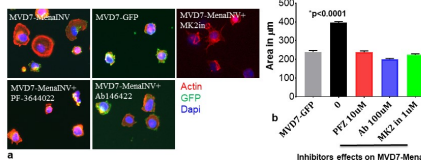


The effect of treatment with 100nM MK2 inhibitor (Celgene), 100nM PF-384022 (Pfizer) and 100nM Ab146422 (AbCam) was evaluated in a fibronectin fibrillogenesis assay using Mena-null fibroblasts, MND7 cell lines engineered to stably express GFP-tagged versions of Mena^{INV} or GFP alone.

(a) Immunofluorescence imaging of fibronectin, DAPI and F-Actin from MND7 derivative cells treated with MK2 inhibitors. Mena-null control (MND7-GFP) and Mena-INV-positive control (MND7-MenaINV). (b) Deposition of fibronectin significantly ($p < 0.0001$) increased in the untreated MND7-MenaINV cells. Fibronectin deposition decreased to MND7-GFP Mena-null control levels following incubation with the 100nM MK2 inhibitor, 100nM PF-384022 or 100nM Ab146422. Data were analyzed using Anova, compared to the MND7-GFP control and plotted as mean with S.E.M (n=4).

Spreading and Adhesion Assay

MK2 inhibitor treatment reversed the Mena-dependent spreading and adhesion phenotype to Mena-null levels

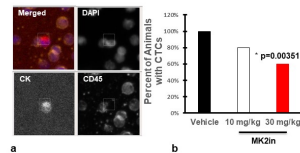


The effect of treatment with 100nM MK2 inhibitor (Celgene), 100nM PF-384022 (Pfizer) and 100nM Ab146422 (AbCam) was evaluated in a spreading and adhesion assay using derivative MND7 GFP-tagged cell lines. (a) Immunofluorescence imaging of GFP, DAPI and F-Actin from MND7 derivative cells treated with MK2 inhibitors. Mena-null control (MND7-GFP) and Mena-INV-positive control (MND7-MenaINV). (b) Spreading and adhesion was significantly reduced ($p < 0.0001$) to Mena-null levels following treatment with the 100nM MK2 inhibitor, 100nM PF-384022 and 100nM Ab146422. Data were analyzed using Anova, compared to the MND7-GFP control and plotted as mean with S.E.M (n=4).

Orthotopic Model of Human Triple Negative Breast Cancer in NOD/SCID Mice

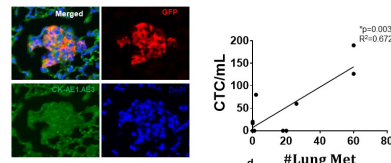
NOD/SCID mice were injected orthotopically into the mammary fat pad with derivative MDA-MB-231 triple negative breast cancer cells and administered the MK2 inhibitor (Celgene) PO QD for up to 55 days.

MK2 inhibitor treatment significantly reduced dissemination of CTCs



Blood was collected and assessed to quantify the number of circulating tumor cells (CTCs) at RareCyte, Inc. using the CyteFinder® Imaging System circulating tumor cell (CTC) separation platform. (a) Immunofluorescence imaging of Cytokeratin (CK), CD45, and DAPI of CTCs. (b) Administration of 30mg/kg MK2 inhibitor to mice implanted with MDA-MB-231-Mena^{INV} cells significantly reduced dissemination of detectable CTCs compared to untreated animals ($p < 0.0351$; p-value comparing the percentages in each treatment group to control is derived from a Fisher's exact test). Four of 10 (40%) mice in the 30mg/kg MK2 inhibitor treatment group had no detectable CTC compared to zero in the control.

Lung metastasis significantly correlated with the number of CTCs

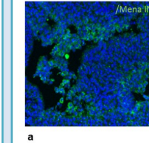


(c) Immunofluorescence imaging of paraffin embedded lung sections from NOD/SCID mice bearing MDA-MB-231-MenaINV xenografts stained for anti-GFP, Cytokeratin and DAPI. Micrometastatic foci were quantified as >5 positively identified tumor cells. (d) Regression analysis showed a significant correlation ($p = 0.0037$) for the number of CTCs/mL of blood and lung metastasis in the MDA-MB-231-Mena^{INV} 30mg/kg MK2 inhibitor treatment group (Pearson's $R = 0.8199$, $R^2 = 0.6722$). Three of 10 (30%) mice in the 30mg/kg MK2 inhibitor treatment group had no metastasis compared to 1/11 (9%) in the control group. For the subset of mice administered 30mg/kg MK2 inhibitor that had no detectable CTCs, the mean number of lung metastasis reduced to 9.6 compared to 44.3 for the untreated control.

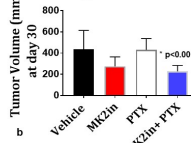
Orthotopic Model of Triple Negative Breast Cancer in FVB Syngeneic Mice

FVB mice were transplanted with 1mm³ tumor pieces derived from MMTV-PyMT mice bearing spontaneous breast cancer tumors and treated with 30mg/kg MK2 inhibitor (Celgene) administered PO QD for 28 days as a single agent or in combination with 10mg/kg paclitaxel IP Q5x3days.

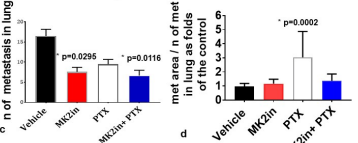
Primary tumors from FVB recipient mice transplanted with MMTV-PyMT tumors were MenaINV-positive



Combination treatment with MK2 inhibitor and Paclitaxel inhibited primary tumor growth



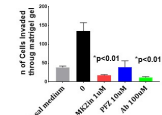
MK2 inhibitor significantly reduced the number of lung metastasis alone and in combination with paclitaxel



(a) MMTV-PyMT primary tumors on FVB mice were Mena-positive by immunofluorescence staining for MenaINV protein isoform expression. (b) Administration of 10mg/kg paclitaxel or 30mg/kg MK2 inhibitor monotherapy had no significant effect on the volume or weight of primary tumors compared to control. Treatment with 10mg/kg paclitaxel in combination with 30mg/kg MK2 inhibitor significantly reduced the volume and weight of primary tumors as compared to control ($p < 0.001$, non parametric t-test, n=6). (c) Treatment with 30mg/kg MK2 inhibitor significantly reduced ($p = 0.0295$) the mean number of lung metastasis compared to the control group. Significant reductions ($p = 0.0116$) in the mean number of lung metastasis were also observed for the 30mg/kg MK2 inhibitor plus 10mg/kg paclitaxel combination group. (c-d) Paclitaxel monotherapy had no significant effect on the number of lung metastasis but did significantly increase the size of lung metastasis ($p = 0.0002$, Dunn's multiple comparison test, n=6).

Boyden chamber invasion assays

MK2 inhibitors reversed migration and invasion phenotype on triple negative breast cancer cells



The effect of treatment with 100nM MK2 inhibitor (Celgene), 100nM PF-384022 (Pfizer) and 100nM Ab146422 (AbCam) was evaluated in a Matrigel Boyden chamber invasion assay using derivative MDA-MB-231 cell lines stably expressing GFP-tagged versions of Mena^{INV} at levels equivalent to metastatic breast cancer *in vivo*. Invasion of MDA-MB-231-Mena^{INV} cells toward EGF was significantly reduced following treatment with 100nM MK2 inhibitor ($p = 0.019$) and 100nM Ab146422 ($p = 0.0149$) compared to the non treated group.

Conclusions

Mena-induced metastatic tumor cell phenotypes were reversed to Mena-null levels when treated with MK2 inhibitors *in vitro*

Treatment with the MK2 inhibitor *in vivo* reduced cancer cell dissemination in two models of aggressive breast cancer

In a syngeneic MMTV-PyMT breast cancer model:

- MK2 inhibitor monotherapy significantly reversed lung metastasis similar to the published effects of Mena deficiency in the MMTV-PyMT murine model

In a MDA-MB-231-Mena^{INV} orthotopic triple negative breast cancer model:

- Dose-dependent reductions in CTCs with significant increases in the number of animals without any detectable CTCs were observed following treatment with the MK2 inhibitor
- Significant correlation of CTCs and lung metastasis in NOD/SCID mice suggest CTCs may be used to monitor the effectiveness of anti-MK2 therapy to block cancer cell metastasis in Mena-positive tumors

MK2 inhibitor reversed resistance of metastatic breast cancer to paclitaxel.

- Paclitaxel significantly increased the size of lung metastasis and had no significant effect on the growth rate of primary tumors or number of lung metastasis
- MK2 inhibitor alone and in combination with paclitaxel therapy significantly reduced the growth of MMTV-PyMT tumors and the development of lung metastasis but not in response to paclitaxel monotherapy

References: "MENA Confers Resistance to Paclitaxel in Triple-Negative Breast Cancer" Oudin M.J. et al *Mol Cancer Ther*. 2017 Jan;16 (1):143-155)

"Neoadjuvant chemotherapy induces breast cancer metastasis through a TMEM-mediated mechanism." Karagiannis G.S. et al *Sci Transl Med*. 2017 Jul 5;9 (397)