MARCKS protein inhibitors attenuate cancer cell migration/metastasis

Methods

In vitro migration assays

For the in vitro migration assays, 1 x 10^5 of three different human lung cancer cell lines (A549, PC-9, and CL-1S) were cultured in Transwell® plates (24-well, 8-µm pore size). Either PBS (control), MANS, or 3 different N-terminal peptide inhibitors analogs of MANS (BIO-11006, BIO-11002, or BIO-11001) were incubated with the cells for 12 hours, and cells that migrated from the upper to lower surface of the chambers were stained with hematoxylin and counted.

MARCKS siRNA studies

PC-9 and A549 cells were seeded in 6-well plates and cultured until cells reached 70% confluence. Cells were transfected with 100nM of MARCKS siRNA or control siRNA (100nM) from Ambion (Austin, TX) using the DharmaFECT Duo Transfection reagent (Dharmacon, Lafayette, CO). After 72 hours, cells were harvested and equivalent amounts of proteins separated by SDS/PAGE for immunoblot analysis. Western blot analysis was performed to confirm siRNA-induced down regulation of endogenous MARCKS via immunoblotting with MARCKS antibody. Blots were re-probed with β-actin as a loading control. MARCKS protein was knocked down ~ 50-60% by MARCKS siRNA compared to cells treated with control siRNA.

In vivo metastasis evaluations

For the first in vivo metastasis evaluations, PC-9 cells were orthotopically-injected into the lungs of NOD-SCID mice with or without pre-incubation with either MANS or BIO-1106, and animals were given peptides after 7 days and every 3 days hence, up until day 25, when the mice were harvested and examined. Specifically, 1 x 10^5 cells were suspended in 100 µL PBS containing 0.5 mg/mL Matrigel. Cells were incubated with appropriate peptides for 3 hours. The mice were anesthetized and the cells directly injected into the left lung of mice with an insulin syringe (29 gauge). Another group of orthotopically-injected NOD-SCID animals received treatment with BIO-1106 as an inhaled aerosol starting at day 4 and every day thereafter up to day 25.

Comparison of effects on tumor metastasis of BIO-11006 given IP or as an inhaled aerosol

SCID mice were anesthetized and PC-9 cells were directly injected into the left lung via an insulin syringe (29 gauge). BIO-1106 peptide was administered either as an inhaled aerosol every day starting at day 4 post-orthotopic injection or by IP injection starting at day 7 post-orthotopic injection. Animals were euthanized at day 25, metastatic tissues harvested. The number of macroscopic metastatic tumor nodules in lungs were counted and recorded. Values are expressed as Mean ± SEM of 7-8 animals in each group. Both the MANS and BIO-1106, with treatment started at either 1 day prior or 3 days post tail vein injection, significantly inhibited metastasis. * = p<0.05; ** = p<0.01.

Conclusions

1. N-terminal peptide inhibitors of MARCKS attenuate migration of human cancer cell lines in vitro in migration chambers in a dose-dependent manner.


3. N-terminal peptide inhibitors of MARCKS significantly attenuate tumor metastasis in these models when administered either via IP injection or as an inhaled aerosol.

4. N-terminal peptide inhibitors of MARCKS may potentially be useful as anti-metastasis therapy in lung and perhaps other cancers.