MECHANISM OF ACTION

STUDIES IN A549 CELLS SHOW THAT MARCKS-INHIBITORY PEPTIDES (IN THIS CASE MANS PEPTIDE) BLOCKS PHOSPHORYLATION OF MARCKS IN RESPONSE TO TWO KNOWN STIMULATORS OF CELL MIGRATION: FETAL BOVINE SERUM (FBS) AND PHORBOL 12 – MYRISTATE 13-ACETATE (PMA)

MARCKS SEQUESTERS THE BIOACTIVE LIPID PHOSPHATIDYLINOSITOL 4,5 – BIPHOSPATE (PIP2) WITHIN LIPID RAFTS IN THE CELL MEMBRANE. WHEN MARCKS IS PHOSPHORYLATED, IT RELEASES PIP2 FROM THESE SEQUESTERED SITES. RELEASED PIP2 THEN CAN ITSELF BE PHOSPHORYLATED, WHICH ALLOWS IT TO INTERACT WITH NUMEROUS SIGNALING PATHWAYS LEADING TO CELL MIGRATION (METASTASIS)

BY BLOCKING PHOSPHORYLATION OF MARCKS, MANS ALSO BLOCKS RELEASE AND ACTIVATION OF PIP2

FINALLY, WE IDENTIFY FOCAL ADHESION KINASE (FAK) AS A PRIMARY DOWNSTREAM TARGET OF RELEASED PIP2 RELATED TO CANCER CELL MIGRATION. MANS INHIBITS PHOSPHORYLATION AND THUS ACTIVATION OF FAK BY BLOCKING PHOSPHORYLATION OF MARCKS
FOCAL ADHESION KINASE (FAK)

FAK IS A KEY REGULATOR OF GROWTH FACTOR RECEPTOR AND INTEGRIN-MEDIATED SIGNALS

• Increased FAK expression is observed in many cancer cells, both primary and metastatic; high expression is associated with a poor prognosis

• FAK promotes malignancy and metastasis via highly – coordinated signaling events that orchestrate a diverse range of cellular processes, including migration and invasion

• The first step in FAK activation is binding with PIP2
Focal Adhesion Kinase
Phosphorylated downstream of PIP2 release and after PIP2 binds to FAK at FERM domain
Role of MARCKS/PIP2 and FAK in Cancer Cell Migration

- MARCKS sequesters PIP2 within lipid rafts in the plasma membrane; FAK signaling is minimal in this situation.
- When MARCKS is phosphorylated, as in cancer cells, PIP2 is released from sequestered sites.
- PIP2 then binds specifically to FAK at the FERM domain, activating FAK via stimulating autophosphorylation at Y397.
- Autophosphorylation at Y397 initiates a series of additional FAK phosphorylations throughout the molecule, such as at Y925, Y397, Y577, etc.
- When FAK is thus activated, the phosphorylated C-terminus of FAK binds to and activates proteins involved in migration: (Talin; Vinculin, Paxillin, Integrins, etc.)
- At the same time, PI3K binds to FAK at the Y397 site and becomes activated, provoking the PI3K/AKT pathway [proliferation, survival events].
Role of MARCKS/PIP2 and FAK in Cancer Cell Migration (2)

Thus, the sequence of activation is:

- MARCKS → P-MARCKS → PIP2 → FAK
  - PI3K → AKT → proliferation, survival
  - C-terminus PO4 → Integrins, Talin, Vinculin, → Cell Migration

- BIO-11006 blocks the initial step in the pathway, the MARCKS → P-MARCKS (phosphorylation of MARCKS)

- Thus, everything downstream of P-MARCKS is inhibited by BIO-11006: MARCKS phosphorylation, PIP2 release, activation of FAK, integrin, talin, vinculin, and thus Cell Migration. Also blocks PI3K/AKT pathway.
EVIDENCE FOR THIS MECHANISM

*Peptide treatment blocks:*

- MARCKS phosphorylation
- PIP2 release from membrane sites
- FAK activation (phosphorylation at Y397 [auto] and Y925; [tyrosine sites on FAK that get phosphorylated])
MANS peptide Inhibits Phosphorylation of MARCKS and FAK in A549 cells

[Note: FBS is used to stimulate cells]
MANS peptide Inhibits Phosphorylation of MARCKS and FAK in A549 cells

[Note: PMA is used to stimulate cells]
MANS peptide inhibits binding of Talin and FAK to PIP\(_2\) (Co-IP with PIP\(_2\))
[Note: FBS is used to stimulate cells]
MANS BLOCKS MARCKS
PHOSPHORYLATION IN RESPONSE TO FBS

• NEXT SLIDE SHOWS, VIA IMMUNOFLUORESCENCE:

• **TOP ROW:** MARCKS CO-LOCALIZES WITH PIP2 IN CELL MEMBRANES
<table>
<thead>
<tr>
<th></th>
<th>PIP2</th>
<th>MARCKS (GFP)</th>
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MANS BLOCKS MARCKS PHOSPHORYLATION IN RESPONSE TO FBS

• NEXT SLIDE SHOWS, VIA IMMUNOFLUORESCENCE:

• MIDDLE ROW: FBS STIMULATES MARCKS RELEASE OF PIP2 (VIA PHOSPHORYLATION OF MARCKS). MARCKS APPEARS TO MOVE TO CYTOPLASM WHILE PIP2 REMAINS IN MEMBRANE
No FBS

FBS

FBS + MANS
MANS BLOCKS MARCKS PHOSPHORYLATION IN RESPONSE TO FBS

• NEXT SLIDE SHOWS, VIA IMMUNOFLUORESCENCE:

• BOTTOM ROW: MANS TREATMENT RESULTS IN MARCKS REMAINING IN THE MEMBRANE, CO-LOCALIZED WITH PIP2 IN CLEARLY DISCRETE SITES
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