Mesothelin promotes mesothelioma cell invasion and MMP-9 secretion in an orthotopic mouse model and in malignant pleural mesothelioma (MPM) patients

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Presenter Disclosure

Elliot L. Servais, MD

The following relationships exist related to this presentation:

No Relationships to Disclose
Mesothelin (MSLN)

- Cell-surface antigen overexpressed in >90% epithelioid malignant pleural mesothelioma (MPM)\(^1\)

- Aggressive cell phenotype *in vitro* & *in vivo* studies\(^2,3\)

- Associated with worse prognosis\(^4,5\)

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Study objectives

- To evaluate the effect of MSLN on malignant pleural mesothelioma (MPM) tumor invasion and locoregional aggressiveness
- To investigate the biologic influence of MSLN expression in a mouse model and human MPM patients
MSLN expression MPM cell lines

- Human biphasic MPM (MSTO-211H) transduced to overexpress MSLN

- Murine MPM (AB12) with natural MSLN expression & shRNA MSLN knockdown
MSLN increases tumor cell migration & invasion *in vitro*

- MSLN did not affect cell proliferation *in vitro* (data not shown)
- MSLN significantly increased migration & invasion through modified Boyden chamber assay
MSLN expression promotes invasion genes and gene sets

• Global gene expression profiles comparing MSTO-211H cells with and without MSLN
  • Illumina microarray platform

• Gene set enrichment analysis (GSEA)\(^1\)
  ➢ Upregulated “MMP-cytokine pathway” (FDR<0.01)

• MMP 2 & 9 are increased in mesothelioma & correlate with poor survival\(^2,3\)

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</table>

MSLN promotes mesothelioma cell MMP secretion

- *In vitro* mesothelioma cell supernatant MMP levels
  - Multiplex bead assay
- MSLN overexpression in human MPM cells (MSTO-211H) increases MMP secretion (**p<0.01**)
- shRNA MSLN knockdown decreases AB12 MMP9 secretion
An orthotopic mouse model of MPM

• Tumor grows along pleural surface

• Encases mediastinal structures

Servais EL. *Curr Protoc Pharmacol.* 2011
MSLN overexpression decreases survival

- MSLN overexpression did not affect MPM tumor progression in vivo
- MSLN overexpression decreases mouse survival

MSLN & MMP associated with local tumor invasion

- Local tumor invasion in the orthotopic murine MPM model
- Mice injected with variable mesothelin expressing tumors
MSLN is associated with MMP-9 expression in MPM patients

- Human MPM tissue microarray (n=139, 729 cores)
- Mesothelin is overexpressed in 90% of epithelioid MPM patients
- Increased MMP-9 expression with higher MSLN expression (p<0.001)
Increased MSLN expression correlates with higher T-stage

- Uniform cohort of stage III MPM patient without prior therapy (n=72)
- Strong MSLN expression in 26% vs. 51% of T2 and T3 tumors, respectively (p=0.05)
Conclusions

- MSLN expression associated with:
  1) MPM invasion both *in vitro* and *in vivo*
  2) Increased matrix metalloproteinase secretion
  3) Decreased survival in an orthotopic mouse model

- MSLN is associated with MMP-9 expression and higher T-stage in pleural mesothelioma patients

- These data indicate that mesothelin is an important factor in the locoregional aggressiveness of MPM
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Elevated serum MSLN predicts decreased survival in mesothelioma

Median survival:
• 22 months if Low MSLN
• 13 months if High MSLN

MPM is a regionally aggressive malignancy

Human MPM Survival: Stratified by serum MSLN Level

Cristaudo A. Clin Cancer Res. 2007
Accurate quantitative bioimaging facilitates biomarker validation

- Tumors monitored *in vivo* by quantitative bioluminescence imaging (BLI) & 3-D MRI volume averaging

- BLI accurate measures tumor burden in the orthotopic mouse model
SMRP correlates with tumor progression

- Serum SMRP detected by Mesomark® ELISA
  - MSKCC clinical chemistry laboratory
- SMRP correlates with tumor progression by BLI and MRI