**BACKGROUND & SIGNIFICANCE**

**METHODS**

**RESULTS**

**CONCLUSION**

- **Epithelial-to-mesenchymal transition (EMT)** plays an important role in physiological embryogenesis as well as tissue repair.
- **EMT** phenotypes contribute to the metastatic conversion of human primary carcinomas.
- **EMT** transition is known to be mediated by transcription factors SNAIL, ZEB-1, or TWIST.
- Overexpression of SNAIL is found in invasive breast cancer associated with metastasis and poor prognosis.
- Several kinase pathways and growth factors are known to upregulate SNAIL expression. However, there is evidence that other regulatory mechanisms exist.

- **Cystatins** are inhibitors of cysteine proteases which allow cancer cells to evade immune response through antigen or complement degradation.
- **Cystatin SN (CST1)** is upregulated in senescence, while **cystatin E/M (CST6)**, is a tumor suppressor frequently inactivated in cancers.
- Recent findings suggest that **cystatins A and D** are involved in **EMT**.
- It is unknown if **CST1** and **CST6** play a role in regulating **EMT** in cancer.

**AIM**

This study was aimed to investigate the potential roles of **CST1** and **CST6** in **EMT** in human breast cancer cells, including in low dose taxol treated cells.

**HYPOTHESIS**

It is hypothesized that overexpression of cystatin would alter the **EMT** signaling in breast cancer cells.

**SIGNIFICANCE**

The results obtained from this study are expected to further our understanding of **EMT** signaling in cancer cells and uncover potential roles of cystatin in clinical application.
Overexpression of CST1 Leads to Altered EMT Signaling

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Data Analysis

• Membranes were imaged with Image Lab™ Software, and BIO-RAD Molecular Imager and ChemiDoc™ XRS+.

• Relative intensities of chemiluminescent signals were compared between empty vector controls, taxol-treated cell lines, cystatin-transduced cell lines, and taxol-treated cystatin-transduced cell lines.

• Analysis was done manually on Image Lab, ImageJ, and Microsoft Excel.

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**Figure 1.** Effect of treatment with low dose taxol on MDA-MB-231 cancer cell lines. Irreversible growth inhibition beginning at 2nM of taxol and cell death at >8 nM of taxol.

**Figure 2.** Western blot analysis of MDA-MB-231 proteins. Increased levels of mesenchymal markers ZEB1, Vimentin, and Snail in taxol treated vector control. Transduction with CST 1 led to decreased levels of snail and ZEB1, when compared with the vector control. Treatment with taxol in CST1 or CST6 transduced cells did not lead to increased levels of snail and ZEB1.

**Figure 3.** Quantification of protein expression of snail, ZEB1, beta-catenin, in Taxol-treated vector or cystatin-transduced MDA-MB-231 cell lines.
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CST1 transduction in MDA-MB231 cells led to reduced protein level of Snail and ZEB1. Low-dose taxol treatment increased expression levels of ZEB1 and Snail in vector control, but not in CST1-transduced MDA-MB231 cells.

### DISCUSSION & CONCLUSION

- The role of cysteine protease inhibitors has become a topic of focus in the development of novel chemotherapeutic agents, although their role in breast cancer remains understudied.

- This study showed that low dose taxol treatment in MDA-MB231 cells led to irreversible growth arrest associated with increased expression of EMT markers SNAIL and ZEB1.

- This study also uncovers that CST1 expression in MDA-MB231 cells led to specific reduced expression of SNAIL and ZEB1 and further suppress the increased expression of these proteins by low dose taxol.

- A limitation of this study was that the experiment was conducted on only one cell line.

- Future studies should aim to apply this hypothesis in additional cell lines and to delineate the mechanism by which CST1 alters expression of transcription factors snail and ZEB1.

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Supplementary Materials

Cystatins in cancer
- Cysteine peptidase inhibitors
- Other proteolysis-independent mechanisms

Biomarkers
Apoptosis
Tumor growth
Tumor invasion
Metastasis
Angiogenesis
Antitumor immune response

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https://www.youtube.com/watch?v=yzCAeDg8fjM