Department of Medicine, Feinberg Cardiovascular and Renal Research Institute

SERBP1: Exploiting RNA-Binding Protein-Mediated PAI-1 Inhibition

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Background

The uPA and tPA are serine proteases that catalyze the activation of plasmin. However, plasminogen activator inhibitor type-1 (PAI-1) is the major inhibitor of both uPA and tPA. 5 to 10-fold increases in PAI-1 are common in the pathogenesis of tissue fibrosis but genetic deficiency of PAI-1 in aged mice has promoted spontaneous cardiac fibrosis. This paradox highlights the overall importance of PAI-1 in fibrosis. SERPINE1 mRNA binding protein 1 (SERBP1) is a mRNA binding protein that binds to PAI-1 mRNA. It binds to the cyclic nucleotide-response sequence in the 3'-UTR of the PAI-1 mRNA and regulates the stability of the transcript. It is proposed that the localization of SERBP1 functions to either stabilize or destabilize the PAI-1 mRNA. However, these mechanisms are not clearly understood so defining SERBP1's role in the pathogenesis of age-dependent cardiac fibrosis may be beneficial for developing future treatments.

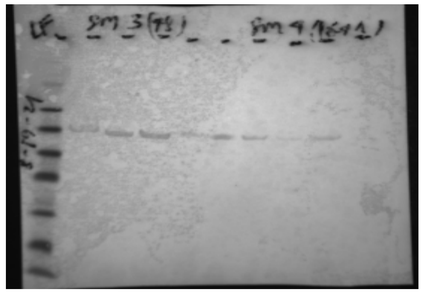
Research Objectives

- Study the functional difference between cytoplasmic and nuclear SERBP1 in human cells and the role of cyclic nucleotides in localization → utilize/develop a treatment that localizes SERBP1 in that location to lower PAI-1 levels
  - Can either be directly through SERBP1 such as targeting the RG/RGG region or indirectly by affecting the cyclic nucleotide
- Study the specific sequence in which SERBP1 binds to the PAI-1 mRNA → develop a protein or cyclic nucleotide that SELECTIVELY binds to this regulatory region

Methods

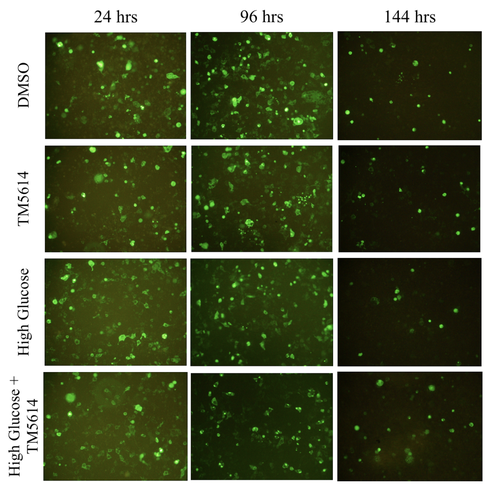
HepG2 cells will be transfected with the GFP-tagged SERBP1 plasmid in order to track the localization of SERBP1 in the nucleus or cytoplasm in response to DMSO, TM5614, High Glucose, and High Glucose + TM5614 treatment. Western blots and qPCRs will be conducted to observe and quantify any differences in PAI-1 and SERBP1 levels between the non-transfected and transfected models. Ultraviolet cross-linking and RNA electrophoretic mobility shift analyses as well as RNAcompete will be performed to measure SERBP1's binding affinity to PAI-1 mRNA as well as study the specific interactions and regions of each transcript in which binding occurs.

Figure 1. PAI-1 Antibody Western Blot Analysis



First four columns represent HepG2 cells treated with DMSO, TM5614, High Glucose, and High Glucose + TM5614 from 48 hours post-treatment while the latter four columns represent HepG2 cells with the same treatments but 144 hours post-treatment.

Figure 2. PAI-1 Localization via Fluorescence



Change in localization 24, 96, and 144 post DMSO, TM5614, High Glucose, and High Glucose + TM5614 treatment as observed by the GFP-tagged SERBP1 plasmid transfected into HepG2 cells. Number of cells expressing more fluorescence in either the nucleus or cytoplasm were counted and compared to attempt to quantify measure of the change in localization due to the specific treatment utilized.

Results

- Very minimal differences observed between PAI-1 and SERBP1 levels in control, TM5614, High Glucose, and High Glucose + TM5614 treatment via Western Blot and qPCR
- DMSO treatment/control demonstrated a shift towards nuclear localization
- TM5614 treatment demonstrated a shift towards nuclear localization
- High Glucose was the only treatment that demonstrated a shift towards cytoplasmic localization
- High Glucose + TM5614 treatment demonstrated a shift towards nuclear localization

Limitations

- HepG2 cell line not ideal for studying cardiac fibrosis
- More qualitative rather than quantitative data would be good, need to quantify the change in localization and PAI-1 levels
- More emphasis needs to be into the effect on PAI-1 → need to match localization effect with effect on PAI-1
- Analysis of binding interactions between SERBP1 and PAI-1 mRNA required to understand and exploit SERBP1's mechanism of action

Conclusions

- Collected localization data contradicts current literature reports in human breast cancer cells: cytoplasmic SERBP1 destabilizes PAI-1 mRNA while nuclear SERBP1 stabilizes PAI-1 mRNA
  - TM5614 should decrease PAI-1 expression → should have localized SERBP1 to cytoplasm
  - High Glucose should enhance PAI-expression → should have localized SERBP1 to nucleus
  - This may be due to the HepG2 cell line → may demonstrate different mechanism of action of SERBP1 in tissue fibrosis
- Potential to further understand and exploit the mechanisms of SERBP1 by studying the specific binding patterns and regions of the PAI-1 mRNA with this mRNA binding protein