The Role of TIMP3 in Regulating Microvascular Endothelial Cell-ECM Interactions and Barrier Function

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BACKGROUND

- Sepsis is a dysregulated host response to an infection. During sepsis, dysfunction of **pulmonary microvascular endothelial cells (PMVEC)** often occurs resulting in a loss of barrier function [1].
- Interactions between PMVEC and the extracellular matrix (ECM) support the stability of the PMVEC barrier [2].
- **Metalloproteinases** are a family of enzymes capable of remodeling the ECM [3].
- **Tissue inhibitor of metalloproteinase 3 (TIMP3)** is an endogenous inhibitor of metalloproteinases. TIMP3 is important for maintaining normal barrier function and TIMP3 levels appear to be decreased under septic conditions [1][3].
- Previous work has demonstrated that a number of cellular processes are altered in *Timp3*$^{-/-}$ PMVEC vs. WT PMVEC under basal conditions, including a decrease in **cell-ECM interactions** [4].

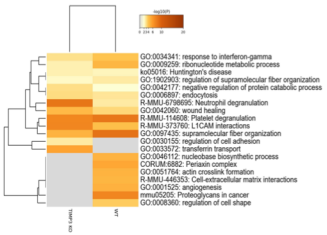

Fig. 1 Enriched Ontology Clusters for *Timp3*$^{-/-}$ and WT PMVEC [4].

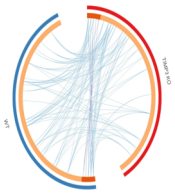

Fig. 2 Gene Overlap Analysis for *Timp3*$^{-/-}$ and WT PMVEC.

- Proteomic analysis, including Terminal Amino Isotopic Labeling of Substrates (TAILS), has identified several peptides to be differentially abundant in *Timp3*$^{-/-}$ PMVEC compared to WT PMVEC [4].

preTAILS	TAILS
↓ Fibronectin	↑ Neogenin
↓ Integrin alpha-5	↑ Exostosin-1
↓ Annexin A1	

Fig. 3 Differentially abundant peptides for *Timp3*$^{-/-}$ PMVEC in comparison to WT PMVEC, as identified by preTAILS and TAILS assay.

OBJECTIVES & HYPOTHESIS

Study Objectives

1. To validate the levels of proteins that were previously identified to be differentially abundant between *Timp3*$^{-/-}$ and WT PMVEC.
   - Fibronectin, Integrin alpha-5, Annexin A1
2. To determine how the abundance of these proteins changes under septic conditions.

Hypothesis

We hypothesize that TIMP3 regulates PMVEC barrier function by promoting PMVEC-ECM interactions.

METHODS

1. PMVEC were collected from *Timp3*$^{-/-}$ mice and WT control mice.
2. Cultured PMVEC were seeded onto a 6-well gelatin-coated plate.
3. Once confluent, cells were treated for 4hr with PBS (basal) or 30 ng/mL cytomix, an equimolar combination of three sepsis-relevant cytokines (interleukin 1β, tumor necrosis factor α, interferon γ).
4. Cells were lysed and the cell lysates were collected.
5. Protein abundance was assessed by western blot.
6. Densitometry was performed using ImageJ and statistical analysis was performed using GraphPad Prism.

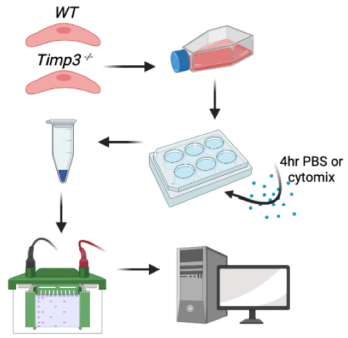

Fig. 4 Flow chart created using Biorender explaining study methods.

RESULTS

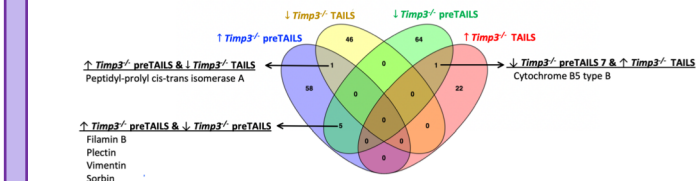

Fig. 5 Venny showing overlap between peptides identified to be differentially represented in preTAILS and TAILS proteomic assay for *Timp3*$^{-/-}$ and WT PMVEC.

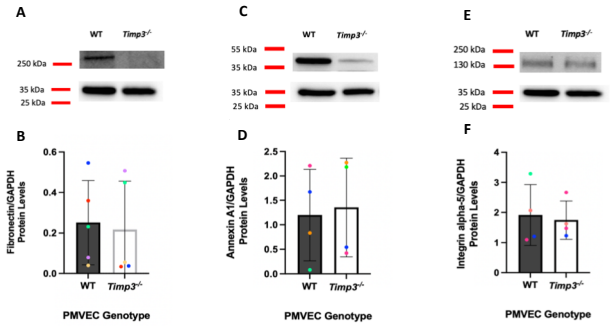

Fig. 6 Western blot showing protein abundance under basal conditions for (A, B) fibronectin, (C, D) annexin A1, and (E, F) integrin alpha-5, relative to GAPDH loading control. There were no significant differences between levels of fibronectin (n=5, P=0.8516), annexin A1 (n=4, P=0.8815) or integrin alpha-5 (n=4, P=0.7878) in WT and *Timp3*$^{-/-}$ PMVEC (paired two-tailed T-test).

RESULTS

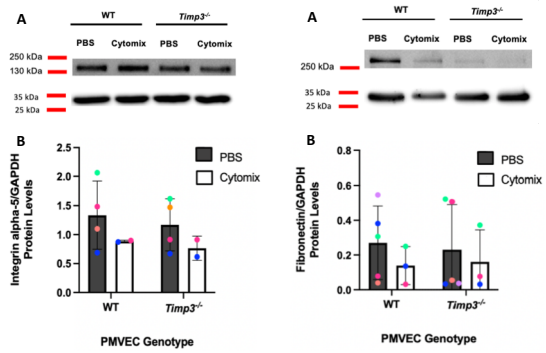

Fig. 7 (A) Western blot and (B) densitometric ratios for integrin alpha-5 from WT or *Timp3*$^{-/-}$ PMVEC after 4hr treatment with PBS or cytomix (n= 2-4). There appears to be no change in integrin alpha-5 levels after 4hr cytomix treatment.

Fig. 8 (A) Western blot and (B) densitometric ratios for fibronectin from WT and *Timp3*$^{-/-}$ PMVEC after 4hr treatment with PBS or cytomix (n= 3-5). There is a trend of decreasing fibronectin levels after 4hr cytomix treatment compared to PBS, in both WT and *Timp3*$^{-/-}$ PMVEC.

FUTURE WORK

- Increase the sample size and continue to validate the abundance of other differentially represented peptides.
- Determine how the amount of fibronectin produced by PMVEC *in vitro* changes over time.
- Explore whether there is altered gene transcription in *Timp3*$^{-/-}$ PMVEC compared to WT PMVEC using qRT-PCR.
- Perform leak studies using trans-PMVEC monolayer Evans Blue-albumin flux to assess if there is a relationship between protein levels and leak.

REFERENCES

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