Reduced CFIm25 Expression as a Regulator of Pulmonary Hypertension Through HAS2

S.D. Collum¹, T. Weng², A. Hernandez³, N.-Y. Chen³, J. Molina¹, J. Amione-Guerra⁴, O. Al-Jabbari², R.R. Bunge⁵, N. Singh⁶, M.R. Blackburn¹, L. Han⁶, H. Karmouty-Quintana³ ¹UTHealth - Houston, TX/US, ²Debakey Heart & Vascular Center - Houston, TX/US

Abstract

Rationale
Cleavage and polyadenylation is an important and regulated step in the maturation of mRNA. Alternative polyadenylation (APA) is typically a shortening of the 3'UTR of affected gene by using a secondary polyadenylation signal. This shortening can remove regulatory elements such as miRNA binding sites and can lead to increased expression of these mRNAs. APA and the resulting expression changes are known to drive the proliferative and tumorigenic properties in some cancers. This APA can be induced by the reduced expression of cleavage factor I especially the 28KD subunit(CFIm25). This leads to the hypothesis that APA due to reduced CFIm25 expression could play a role in the proliferative remodeling of pulmonary vasculature seen in pulmonary hypertension (PH).

This research was conducted with human samples and animal models of PH. The human samples included lung and pulmonary artery samples of normal patients and patients with idiopathic pulmonary fibrosis with and without secondary PH as well as pulmonary arterial hypertension (PAH). The animal model used was the hypoxia-Sugen5416 mice as well as the bleomycin model. Western blot and immunohistochemistry methods were used to monitor protein expression changes while qRT-PCR and RNA-seq monitored mRNA level changes.

Figure 1
Depletion of members of the CF Complex leads to APA. In general, genes whose 3'-UTRs undergo APA have a 3'UTR that is shorter and which depletes HA precursors and inhibits HAS. 4MU also reduces the pulmonary hypertension symptoms seen in the bleomycin model of PH.

Figure 2
Pulmonary artery smooth muscle cells (PASMCs) from patients with COPD-PH show lower levels of CFIm25 expression compared to normal. Hypoxia, which can induce remodeling seen in PH reduces these levels further. Patient samples from IPF-PH and PAH show reduced expression of CFIm25 compared to control and IPF alone in IHC.

Figure 3
CFIm25 expression is reduced in the pulmonary artery of patients with PAH as well as IPF and IPF-PH.

Figure 4
CFIm25 expression is lower in the pulmonary artery of mice treated with hypoxia and VEGFRI antagonist Sugen 5416.

Figure 5
After KD of CFIm25 in human PASMCs resulted in the significant shortening of 86 3'UTRs as measured by RNAseq. Among the genes with shortened 3'UTRs was HAS2 a gene responsible for the production of the extracellular matrix component hyaluronan(HA). This shortening and increased expression at the RNA level was verified by qRT-PCR.

Figure 6
HAS2 expression correlates with increased mPAP in patients with IPF. Suggesting a link between HA production and PH.

Figure 7
IHC staining for HA shows excessive production surrounding remodeled vessels in patients with PH secondary to IPF.

Figure 8
Increased HA production after bleomycin treatment is reduced by 4-MU, which depletes HA precursors and inhibits HAS. 4-MU also reduces the pulmonary hypertension symptoms seen in the bleomycin model of PH secondary fibrosis.

Figure 9
4-MU treatment does not reduces the fibrosis that is induced by bleomycin treatment. Suggesting that HA is involved in the development of PH but not in the development of fibrosis.

Conclusions
1. CFIm25 levels are reduced in patients with PH and in animal models of PH.
2. This reduced expression of CFIm25 leads to APA in a number of genes including HAS2.
3. Increased HAS2 expression leads to increased production of HA and correlates with PH symptoms.
4. Blocking HA production through HAS2 both pharmacologically or transgenically reduces PH symptoms in mouse models.

Future Directions
We will verify that loss of CFIm25 in mouse models of PH causes development of the symptoms that are reduced by inhibition of HA production. We will also identify APA in HAS2 of patients with PH.