Snake Venom Cystiene-Rich Secretory Proteins induced the up regulation of Caveolin-1 Expression in human endothelial cells

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Introduction

Snake venom Cysteine rich secretory proteins (svCRiSPs) are widely distributed in the venoms of many species of snakes. They are non-enzymatic proteins with molecular weights of 26-28 kDa. Studies revealed that svCRiSPs can inhibit smooth muscle contraction, cyclic nucleotide-gated ion channels, angiogenesis, and induce vascular permeability and proinflammatory responses. We found that both Css-CRiSP isolated from Crotalus scutulatus scutulatus and App-CRiSP isolated from Agkistrodon piscivorus piscivorus enhanced caveolin-1 expression in HDBECs using reverse phase protein microarray analysis. While the expression of proteins and phosphorylation involved in the JNK and Akt pathways, adherens junctions' integral membrane protein, and cell adhesion molecules were significantly altered in HDLECs treated with these svCRiSPs. The aim of the study is to confirm the changes in potential signaling of Caveolin-1 in HDBECs and HDLECs by western blot analysis.

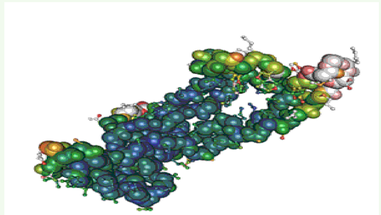
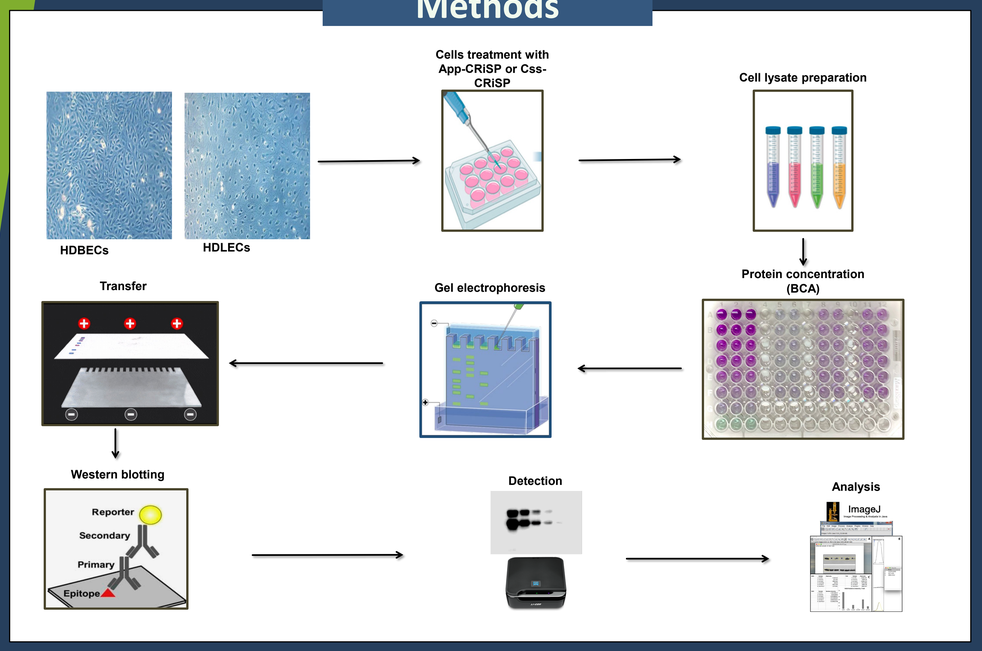


Figure 1: Cav-1 regulate signaling molecules in cell membranes and ranges from 21-24 kDa

Methods



Results

The effect of Css-CRiSP an App-CRiSP on signaling pathway HDLECs and HDBECs

	HDBEC	HDLEC
Css-CRiSP	Caveolin-1, mTOR-p2448	PLC gamma , N-Cadherin
App-CRiSP	Caveolin-1, Akt-pS473, Src-PY416	JNK_pT183-Y185

Here we see the signaling pathways of HDLECs and HDBECs by Css-CRiSPs and App- CRiSP

On HDBEC both App and Css-CRiSP express enhanced Caveolin-1, also PLC and N-Cadherin are significantly altered in HDLEC.

Western Blot analysis

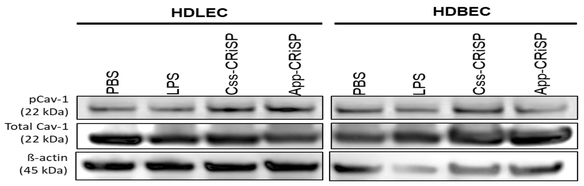


Figure 2: Western blot of svCRiSPs in HDLEC and HDBEC for 1 hour with anitbodies of B-actin, pCav-1, and total cav-1

The work that has been conducted was to test in confirming the expression of caveolin 1 signaling pathways on HDLECs and HDBECs via application of Css-CRiSP and App-CRiSP.

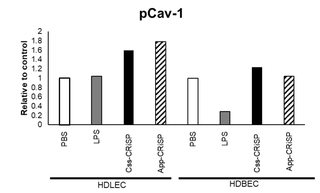
Cell permeability activity

In Cav-1 for HDBEC, Css-CRiSP and App-CRiSPS had a higher density then compared to the control (Fig A). In HDLEC only Css-CRiSP showed a higher increase compared to the control (Fig B.).

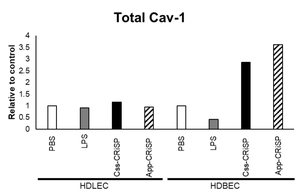
Only in HDLEC did Css and App-CRiSP had a higher increase in density

In phospho-Caveolin was adjusted with B-actin, both Css and App-CRiSP showed higher density in comparison to the control (Fig C)

A)



B)



C)

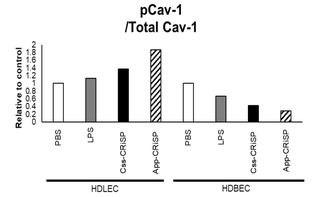


Figure A: Phospho-caveolin that has been adjusted with ß-actin

Figure B: Total caveolin that has bee adjusted with ß-actin

Figure C: Results of ratio activated proteins versus non-activated proteins

Conclusions-Future work

- Css-CRiSP & App-CRiSP treated HDLECs and HDBECs showed greater density in Phospho-Caveolin and higher density when compared to caveolin in HDLEC relative to the control.
- Further studies are required to confirm the intracellular mechanisms through which Css-CRiSP & App-CRiSP reaches to provoke vascular permeability. Verify the expression of these signaling molecules using other immunochemical assays

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