Discovery of Small Molecule Inhibitors Targeting the HEG1-KRIT1 Protein-Protein Interaction with Deep Convolutional Neural Network

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ABSTRACT

The Kruppel-like factors 4 and 2 (KLF4/2) are transcription factors that play essential roles in endothelial cell phenotype and vascular homeostasis. KLF4/2 regulates the expression of factors responsible for antithrombotic and anti-inflammatory effects in endothelial cells. Therefore, an increased level of endothelial KLF4/2 has a vasoprotective effect. Genetic studies demonstrated that the suppression of endothelial KRIT1 (Krev interaction trapped protein 1) or HEG1 (Heart of glass 1) results in upregulation of KLF4/2. Besides, HEG1 serves as an endothelial cell membrane anchor for KRIT1 and is essential for KRIT1 function. Thus, we hypothesize that pharmacological manipulation of the HEG1-KRIT1 interactions could lead to upregulation of KLF4/2, promoting vasoprotection. We applied the AtomNet® model, a deep convolutional neural network for structure-based drug discovery, to screen 2.5 million commercially available compounds against the HEG1-KRIT1 interface. With an unbiased, AI-guided selection process, we tested 94 compounds in vitro and identified 2 hits. Target engagement experiments demonstrated that both compounds blocked HEG1-KRIT1 interaction in a dose-dependent manner with flow cytometry and fluorescence polarization assay. We continue to harness the power of AtomNet® technology for the hit-to-lead effort.

BACKGROUND

HEG1

- HEG1 is an essential regulator of vassal formation stabilizing the endothelial cell junctions.
- HEG1 is a membrane anchor for KRIT1 and is essential for KRIT1 function.

KRIT1

- KRIT1 suppress the expression of KLF4/2 which regulates vascular homeostasis and flow-mediated vasoprotection.

Hypothesis

- Pharmacological manipulation of the HEG1-KRIT1 interactions could lead to upregulation of KLF4/2, therefore induce vasoprotection.

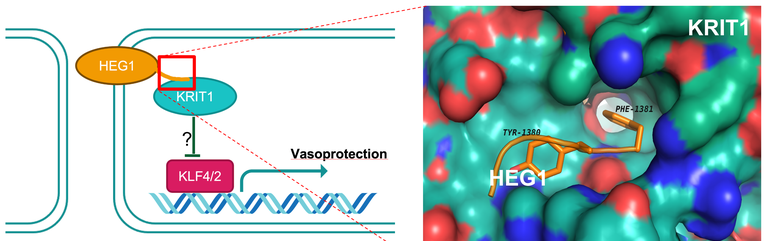
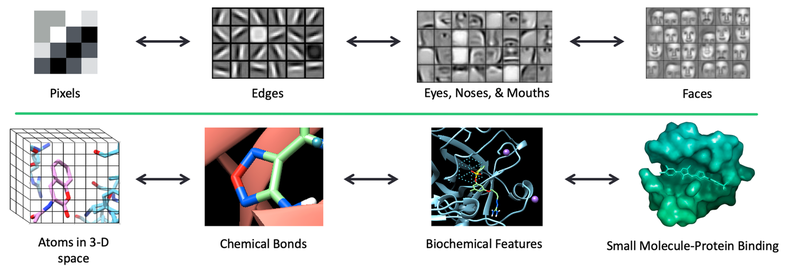


Figure 1: Biological hypothesis of targeting HEG1-KRIT1 interactions for Vasoprotection

METHODS

AtomNet® Virtual Screening



AtomNet® model – Deep Convolutional Neural Networks applied to drug discovery

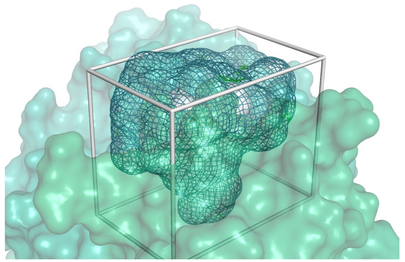


Figure 2: The screening site on KRIT1

To identify novel inhibitors targeting HEG1-KRIT1 protein-protein interaction, AtomNet® model was utilized to score a library of 2.5 millions commercially available small molecules. A set of 94 compounds were selected with an unbiased, AI-driven process, and made available for experimental validations.

Assays

Effect of Atomwise compounds on HEG1-KRIT1 protein-protein interactions were evaluated using 2 assays:

- **Flow Cytometry Assay**
  - Primary screen: All virtual hits at single concentration (30μM)
- **Fluorescence Polarization Assay**
  - A 7-mer tracer was designed and synthesized based on the C-terminus of KRIT1
  - An orthogonal assay to validate hits identified from the primary screening

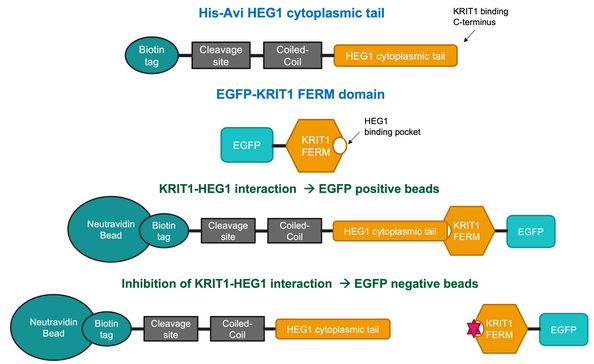


Figure 3: Flow Cytometry assay used to evaluate Atomwise's predicted compounds

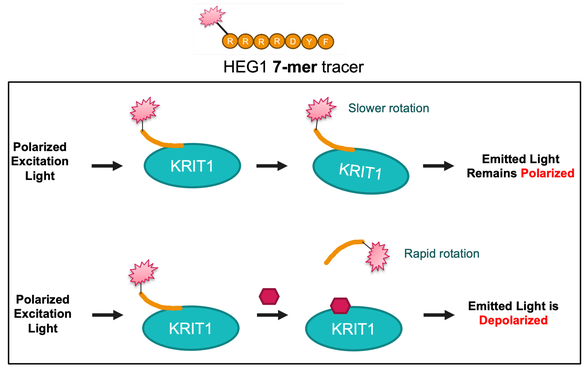
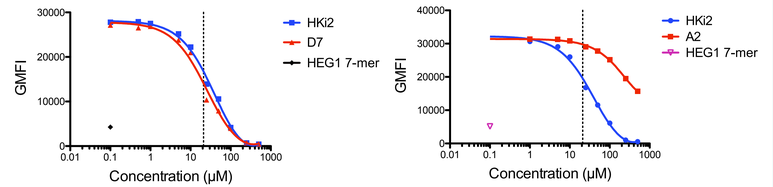


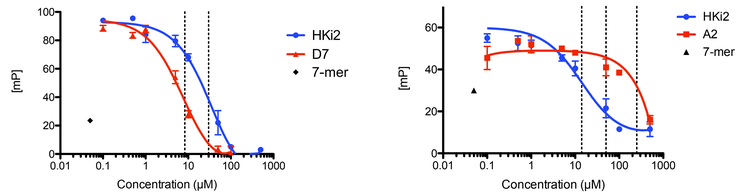
Figure 4: Fluorescence polarization assay used to evaluate Atomwise's predicted compounds

RESULTS

Flow Cytometry Assay



Fluorescence Polarization Assay



	HKi2 (control)	D7	A2
FP (IC50)	33 μM	8 μM	249 μM
FP (Ki)	10 μM	2 μM	75 μM
Flow cytometry (IC50)	30 μM	21 μM	250 μM

CONCLUSION

- Two hits were identified from the pilot screening against HEG1-KRIT1 protein-protein interactions.
- AtomNet® technology demonstrates its capability of identifying hits targeting protein-protein interaction with unprecedented binders.
- We continue to harness the power of AtomNet® technology to guide the hit expansion process to improve potency.