McMaster University/[1]Faculty of Health Science/[2]Department of Pathology and Molecular Medicine

Identification of a novel anti-antithrombin RNA aptamer

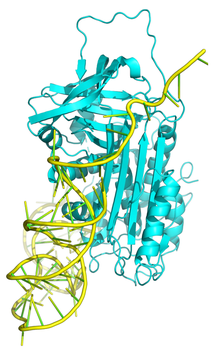
Mostafa Hamada[1], William Sheffield[2]

INTRODUCTION

- Antithrombin-III (AT) is the most abundant anticoagulant in human plasma, circulating at levels of 140 ug/mL (2.5 uM)[1].
- Naturally, AT targets thrombin, factors Xa, Va, and XIIa, with the highest specificity to thrombin.[2]
  - Inhibition of these proteases is significantly increased in presence of heparin.[2]
- Patients experiencing AT-deficiency have illustrated evidence of thrombosis and blood clots, indicating that clotting is promoted.[3]
- In cases of hemophilia or traumatic blood loss, removing blockages imposed on the coagulation cascade is a vital step. Therefore, by inhibiting AT, blood clotting can be accelerated.

AIMS

- Develop a modified RNA aptamer with a high binding affinity to AT
- Develop a therapeutic RNA aptamer capable of blocking AT inhibition of thrombin.



METHODS

- We used systematic evolution of ligands by exponential enrichment (SELEX) for 10 irritative rounds of selection to enrich the starting RNA library.
- AT was immobilized on an antibody-magnetic bead complex

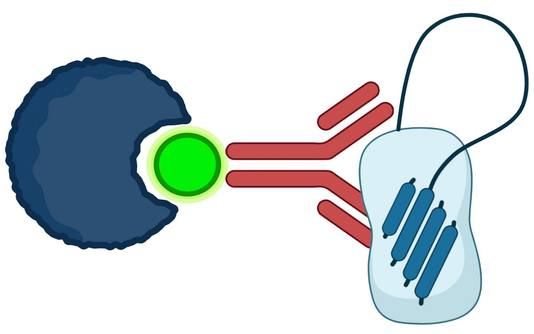


Figure 1: Beads-antibody-protein complex for immobilizing antithrombin for in-vitro selection

- Identified sequences with the highest read counts in all selected pools by using High Throughput Screening (HTS)
- Chromogenic and aPTT assays utilized for quantifying antithrombin's inhibition of thrombin.
- In silico approaches ranked aptamers based on their thermodynamic binding potential to antithrombin

RESULTS

- HTS identified over 10 aptamer sequences which predominantly populated the selection pool.
- Increased cleavage of chromogenic substrate by thrombin, indicated lower AT activity when incubated with R6_15 and 19.

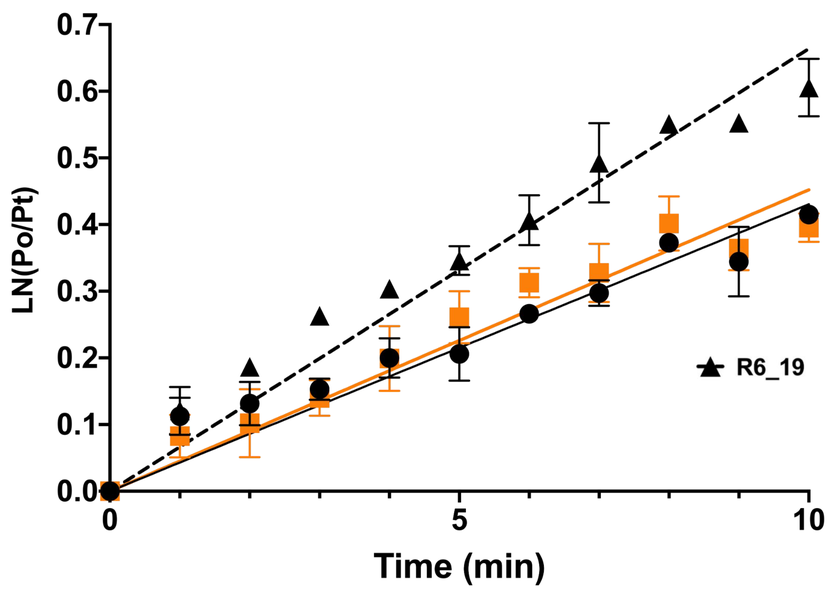


Figure 2: k_{obs} determination of top aptamer candidate R6_19 compared to controls.

- Clotting assays further supported chromogenic assay findings, by indicating that aptamers R6_15 and R6_19 are the most potent binders to AT.
  - Lower clotting times represent lower inhibition of coagulation factors (eg. FVa and thrombin), which means low AT activity

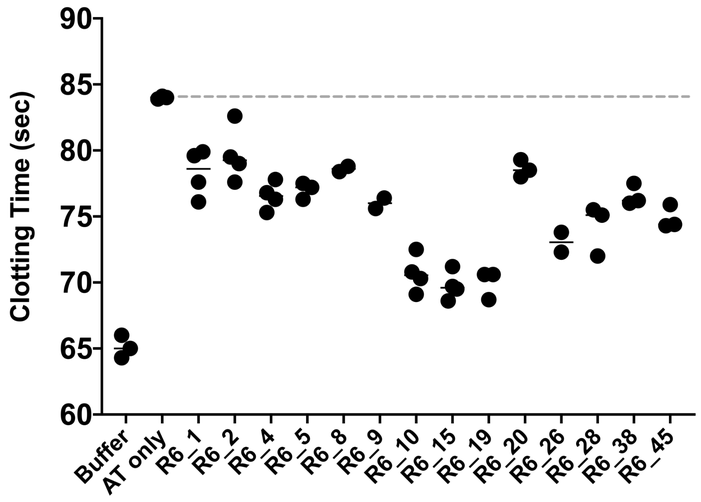


Figure 3: Decrease in clotting time observed when adding aptamers, with the most effect seen by R6_15 and 19.

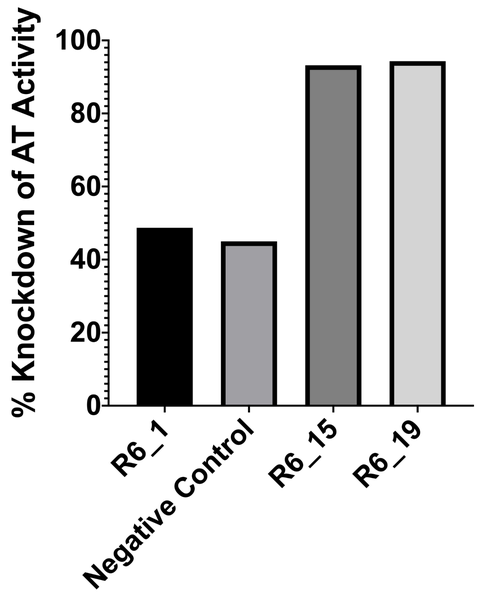


Figure 4: Greater knockdown of AT activity observed in the aPTT assay by adding aptamer R6_15 and 19.

CONCLUSIONS

- In-vitro testing shows significant inhibition of AT by the selected aptamers
- Binding affinity needs to be established.
- In-vivo testing required to make certain of aptamers R6_15 and R6_19 inhibition of AT in a bleeding mouse model

REFERENCES

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2. Tsiang, M., Jain, A.K., Gibbs, C.S. (1997). Functional requirements for inhibition of thrombin by antithrombin III in the presence of absence of heparin. Journal of Biological Chemistry, 272(18):12024-12029. https://doi.org/10.1074/jbc.272.18.12024.
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