4

Transient gene therapy to decrease the stability of thrombi for coagulopathy and thrombosis

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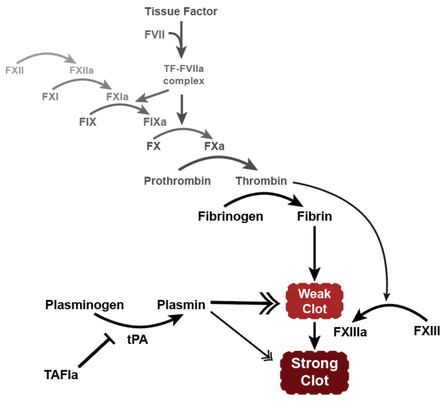
Abstract

Inhibiting FXIII could weaken thrombi without impairing coagulation

Coagulation factor XIII acts in the last step of clot formation, as a transglutaminase, to crosslink fibrin together and to antifibrinolytic molecules. This crosslinking stabilizes blood clots and increases their resistance to fibrinolysis, clot degradation by the activated protease plasmin. Anticoagulants target FX or Thrombin upstream in the coagulation cascade, inhibiting clot formation and posing a risk of excessive bleeding. Targeting FXIII would provide a strategy to reduce the stability of clots while maintaining hemostasis after injury.



Select reactions in the coagulation cascade that lead to the development of a firm clot, resistant to fibrinolysis by plasmin.

Method

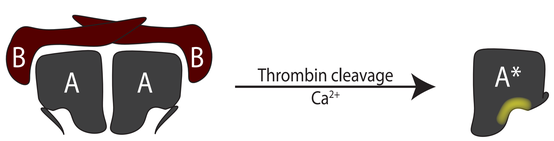
Lipid nanoparticles to deliver siRNA to knockdown the B subunit of FXIII

Current small molecule inhibitors are limited by low specificity and short circulating half-life, making them unsuitable for in vivo use.

siRNA has recently been approved as a therapeutic which can confer many bennifits, including:

- high specificity for the targeted protein mRNA
- long lasting efficacy on the order of weeks
- easy reversibility by plasma or protein transfusion

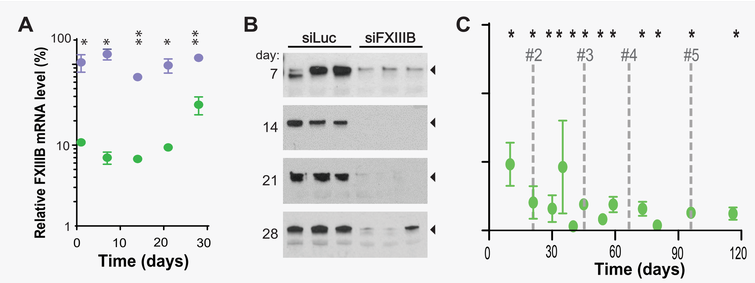
Lipid nanoparticles (LNPs) have been optimized and approved by the FDA for the delivery of siRNA therapeutics. LNPs preferentially accumulate in the liver, and are thus most suitable for targetting proteins synthesized in hepatocytes, such as the B subunit of FXIII (FXIIIB). While the A subunit (FXIIIA) has the enzymatic activity, FXIIIB acts a carrier subunit, extending the circulating half-life of FXIII to over one week.



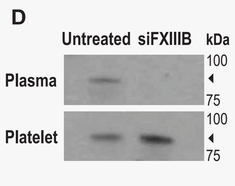
FXIII as it circulates in plasma ($FXIIIA_2B_2$ heterotetramer) before it is activated to a transglutaminase (FXIIIA*).

Results

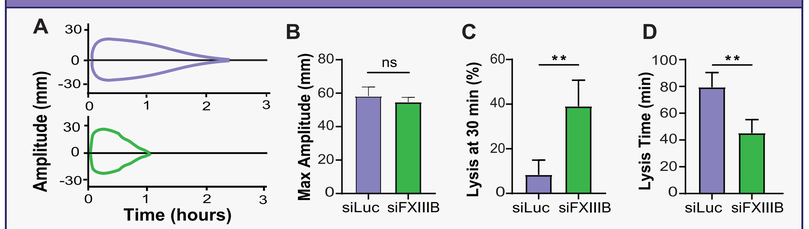
In plasma, but not platelets, siRNA targeting FXIIIB (siFXIIIB) results in long-term reduction of FXIIIA



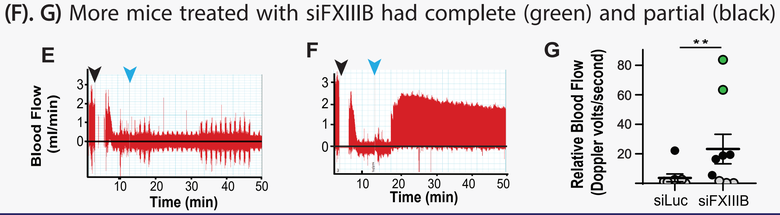
A) FXIIIB mRNA concentration, measured by qPCR, in liver tissue is reduced by 90% for 3 weeks following a single administration of siFXIIIB in LNPs compared to control siRNA-Luciferase. **B)** Western blots against FXIIIA (arrow) show reduced concentration for weeks after administration **C)** FXIIIA depletion can be prolonged indefinitely by repeat dosing at three week intervals. **D-E)** Isolated platelets show steady concentrations of FXIIIA, 14 days after siFXIIIB administration. All data shows the mean ± SEM; * indicates a P-value < 0.05, ** indicates P < 0.01; N = 3 mice per each data marker.



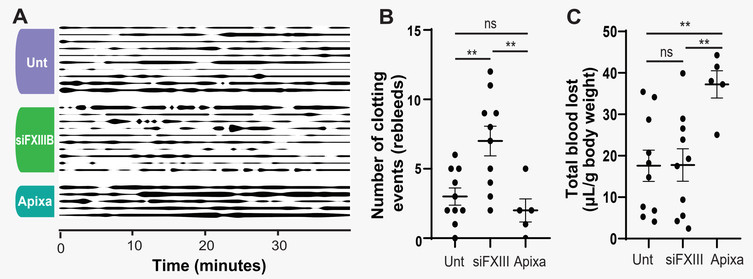
Depletion of FXIIIA enhances fibrinolysis in vitro and in vivo in $FeCl_3$ stimulated arterial thrombosis



A) Thromboeslatography shows more lysis in blood from mice treated with siFXIIIB (green) compared to control siLuc (lavender). While clot stiffness is unchanged **(B)**, percent of clot lysed after 30 minutes is greater **(C)**, and time until complete clot lysis is less **(D)**. In vivo, Doppler ultrasound measured blood flow through the carotid artery after administration of ferric chloride (black arrow) and subsequent injection of tPA (blue arrow) in mice treated with siLuc **(E)** or siFXIIIB **(F)**. **G)** More mice treated with siFXIIIB had complete (green) and partial (black)



Despite reduced clot stability, hemostasis is maintained in a tail transection model of bleeding



Tails of mice pretreated with apixaban (Apixa), siFXIIIB (siFXIII), or untreated (Unt) were transected and the subsequent bleed was observed over a period of 40 minutes. **A)** Graphical representation of bleeding from the wound, qualitatively observed over 40 minutes at 1 minute intervals, shows a greater number of clotting events **(B)**, defined as a cessation of bleeding for 20 seconds or longer. **C)** The total volume of blood lost relative to the individual mouse's weight, quantified by measuring hemoglobin, was highest in the Apixaban group, and unchanged in the siFXIIIB group, compared to untreated.

Summary

- siRNA targeting FXIIIB decreases the concentration of plasma FXIIIA for over 3 weeks following a single injection, and can be prolonged indefinitely with repeat dosing.
- Platelet FXIIIA is not effected by FXIIIB knockdown.
- FXIII depletion increases clot susceptibility to fibrinolysis, without changing clot stiffness, or clot formation time.
- Clots are more failure prone when FXIII is depleted, but bleeding remains less than with anticoagulant therapy.

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