A novel rationale for targeting FXI: Insights from the hemostastic miRNA targetome for emerging anticoagulant strategies

Authors:

Jamie Nourse[1,2] and Sven Danckwardt[1,2,3]

Affiliations:

[1] Center for Thrombosis and Hemostasis (CTH), University Medical Center of the Johannes Gutenberg University, Mainz, Germany

[2] Institute for Clinical Chemistry and Laboratory Medicine, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

[3] German Center for Cardiovascular Research (DZHK), Rhine-Main

Corresponding author:

Sven Danckwardt, MD

University Medical Centre of the Johannes Gutenberg University

Langenbeckstrasse 1

55131 Mainz

Germany

Phone +49 6131 176041

Email sven.danckwardt@unimedizin-mainz.de

Abstract

The treatment and prevention of thrombosis is currently under a period of rapid change with the replacement of traditional anticoagulant vitamin K antagonists, which impair the biosynthesis of a wide range of blood coagulation factors, with direct oral anticoagulants (DOACs), which specifically target factors FXa or FIIa.

Nevertheless therapeutic targeting of blood coagulation is an inherently difficult task as it interferes with the delicate balance of pro- and anticoagulant activities. Although anticoagulants are employed in millions of thrombophilic patients worldwide each year, for a growing population with comorbidities who exhibit an increased risk of bleeding with DOAC treatment satisfying therapeutic options are still lacking and the quest for novel therapeutics continues.

Recently the targeting of factors FXI and FXII have emerged as new therapeutic strategies. As these factors play an important roles in thrombosis, however are more or less functionally dispensable for hemostasis, they may potentially overcoming the functional obstacle of treating or preventing thrombosis without affecting hemostasis.

A key question in following up this approach is which of these two factors is likely to be the more reliable target? Here we present and discuss a hitherto unrecognized rationale for the therapeutic targeting of FXI. This is based on mimicking endogenous FXI gene expression control by therapeutic delivery of miRNA mimics.

Studies are urgently needed to test this therapeutic principle in a clinical setting. This is particularly applicable in hemostaseology where monitoring of therapeutic effects remains a daily routine and thus assessment of the efficacy and safety of such therapeutic components could be simply implemented ushering in a novel therapeutic era with broad applicability.

Keywords

Dabigatranetexilat (Pradaxa), Argatroban (Argatra), Apixaban (Eliquis), Rivaroxaban (Xarelto)

Introduction

Therapeutic targeting of blood coagulation is an inherently difficult task as it interferes with the delicate balance of pro- and anticoagulant activities. Anticoagulants are employed in millions of thrombophilic patients worldwide each year to limit the otherwise essential function of blood clotting[1]. However this intervention inevitably leads to an elevated risk of undesired bleeding[2]. While some bleeding events are clinically mild and less critical, others can be life threatening, such as intracranial or abdominal bleeding.

The dichotomy of therapies targeting the hemostatic system is also evidenced in hemophilic coagulopathies, where blood coagulation is insufficient. In these cases the treatment of acquired or inherited hemophilia requires substitution of the lacking blood coagulation factor(s), ideally without tilting the hemostatic system too far towards a procoagulant state with eventual predisposition to thrombosis[3].

The treatment and prevention of thrombosis is currently under a period of rapid change. Direct oral anticoagulants (DOACs), which specifically target blood coagulation factors FXa or FIIa, are replacing traditional anticoagulants, such as vitamin K antagonists which impair the biosynthesis of factors FII, FVII, FIX and FX (Figure 1A). Nevertheless, satisfying therapeutic options are still lacking for a growing population with comorbidities who exhibit an increased risk of bleeding with DOAC treatment. These include patients with moderate to severe renal failure, hepatic failure, mechanic heart valve or patients under therapy with CYP3A4 and P-Glycoprotein inhibitors[4-7]. This elevated risk primarily reflects interference with metabolic turnover or excretion mechanisms of the DOAC.

Results and Discussion

With great interest we took note of the recent article by Fredenburgh and coworkers[8], which continues and extends a series of articles documenting the targeting of factors FXI and FXII as increasingly evolving new therapeutic strategies. It is hoped that this approach may overcome functional obstacles in the quest for the 'ideal anticoagulant', that of treating or preventing thrombosis without affecting hemostasis. The rationale behind this approach is that both components, FXI and FXII, belong to the so-called "contact system", which appears to play an important role in thrombosis, however they are more or less functionally dispensable for hemostasis[9-14] (Figure 1A). A key question in following up this approach is which of these two factors is likely to be the more reliable target in the challenging endeavor of inhibiting blood clotting while maintaining hemostasis?

Based on the data generated in a recent large-scale determination of functional miRNAs targeting the hemostatic system in the search for novel and rationale therapeutic targets[15], we discover FXI to be targeted by the largest number of miRNAs, while FXII does not appear to be regulated by miRNAs (Table 1). This observation is consistent with further miRNAs reported to target FXI[16-18], while no reports are found for factor FXII. Biologically, this finding is remarkable. It suggests that FXI expression is under tight control via 3'UTR mediated regulation to ensure modest levels of FXI protein while FXII is not. This is in line with the narrower range of FXI plasma levels as compared to FXII (Figure 1B). The biological importance is corroborated by the functional consequences in patients where elevated FXI levels predispose to thrombosis[19-21], while patients with FXI deficiency are protected from venous thromboembolism (VTE) and ischemic stroke[22]. Further support for the regulatory importance of the FXI 3'UTR comes from a recent study in which 3'UTR single nucleotide polymorphisms were found to be associated with plasma FXI activity and risk of venous thrombosis[16]. Remarkably while the FXII 3'UTR is relatively short (~150 nucleotides; nt) the FXI 3'UTR spans over ~1000 nt and, in addition to the

high number of miRNAs, also binds numerous RNA binding proteins (RBPs) in close proximity to, and sometimes even in the direct target region of miRNAs (Figure 1C).

With regards to the important question of Fredenburgh et al., as to which of the two components of the upstream intrinsic pathway (FXI or FXII) is likely to be the better therapeutic target, our data suggests that the specific miRNA mediated control of FXI may be a hitherto unrecognized rationale for the therapeutic targeting of FXI and additionally provides an avenue for this targeting.

While a number of FXI inhibitors, including polypeptides, active site peptidomimetic inhibitors, allosteric inhibitors, antibodies, aptamers and antisense oligonucleotides (ASOs), have been proposed[23], it is important to realize that by mimicking the endogenous function of miRNAs to limit excessive expression, therapeutic miRNA delivery may possibly reflect a more physiological state in limiting FXI levels to treat and prevent VTE while retaining sufficient hemostasis for the prevention of bleeding. Although miRNA targeting to silence FXI could potentially have off-target effects, the functional synergy of several targeting miRNAs in a therapeutic cocktail allows for an increase in effect size and a reduction in side effects[15]. Finally and most importantly, miRNA-mediated targeting may also preserve cell intrinsic regulatory mechanisms, such as modulated 'occlusion' of miRNA binding sites by RNA binding proteins (RBPs)[24-26], ensuring the normal physiological fine tuning of the availability of the factor is maintained[27], Figure 1D). Thus even in the presence of a therapeutic cocktail of miRNAs RBP-mediated regulation may be in place to evade miRNA-mediated silencing for example in order to stock pile blood clotting factors in an acute event of blood loss.

We thus fully concur with Fredenburgh et al. that despite the impressive 'juggernaut' of anticoagulants, there remains room for improvement. miRNA based therapeutics, currently emerging as attractive therapies in many other disciplines[28], may represent an avenue for correction of de-regulated hemostasis and associated processes in the future. Given the

plethora of miRNA based therapeutic possibilities, such as miRNA mimicking, silencing, target specific miRNA target site blockers, knowledge of the miRNA targetome in the hemostatic system is potentially a major trove for future targeted therapeutics, not only for the development of safe anticoagulants but also for developing treatments for coagulopathies. In aid of these endeavors the first comprehensive hemostatic miRNA targetome is provided as a repository accompanying our recent paper[15].

Future studies are urgently needed to test this therapeutic principle in a clinical setting. This is particularly applicable in hemostaseology where monitoring of therapeutic effects remains a daily routine and thus assessment of the efficacy and safety of such therapeutic components could be simply implemented - towards a novel therapeutic era with broad applicability.

Acknowledgments

This work was supported by grants from the BMBF, DFG, DGKL and the Hella Bühler Prize for cancer research.

Contribution

All authors contributed to the research and edited the manuscript; J.N. performed the data analysis; J.N. and S.D. wrote the manuscript.

Disclosure of Conflicts of Interest

The authors declare no competing financial interests.

Table 1. Functional miRNAs targeting the hemostatic system. The first column indicates miRNAs identified from the literature to directly target the gene 3'UTR of blood coagulation factors indicated. The second column is based on miRNAs identified in Nourse et. al.[15] by miTRAP RNA pull-downs and validated by miRNA-mimic-rescue luciferase assays. The final column contains potential miRNAs identified in Nourse et. al[15] by miTRAP RNA pull-downs alone.

Gene	Gene Description	Total reported directly targeting miRNAs	Total directly targeting miRNAs	Potential targeting miRNAs
F11	**Coagulation factor XI**	**14**	**10**	**126**
F8	Coagulation factor VIII	7	7	172
SERPINA10	Protein Z-Dependent Protease Inhibitor (ZPI)	7	7	122
PROZ	Protein Z	6	6	57
SERPIND1	Heparin co-factor II	6	7	74
PLG	Plasminogen	5	5	128
FGA	Fibrinogen alpha chain	4	2	225
FGG	Fibrinogen gamma chain	4	4	127
F7	Coagulation factor VII	2	2	103
PLAT	Plasminogen activator tissue type (tPA)	2	0	76
SERPINC1	Antithrombin III	2	2	57
GP1BA	Glycoprotein Ib platelet alpha subunit	2	0	0
FGB	Fibrinogen beta chain	1	0	145
KLKB1	Kallikrein B1	1	1	48
ADAMTS13	ADAM metallopeptidase with thrombospondin type 1 motif 13	1	0	0
PROC	Protein C	0	0	79
F12	**Coagulation factor XII**	**0**	**0**	**0**
F13B	Coagulation factor XIII B polypeptide	0	0	0
F2	Coagulation factor II (thrombin)	0	0	0
F5	Coagulation factor V	0	0	0
HABP2	Hyaluronan binding protein 2	0	0	0
HRG	Histidine-rich glycoprotein	0	0	0
KNG1	Kininogen 1	0	0	0
SERPINF2	Alpha-2-antiplasmin	0	0	0

References

1. Wendelboe AM, Raskob GE. Global Burden of Thrombosis: Epidemiologic Aspects. *Circulation Research*. 2016;118(9):1340–1347.
2. Eikelboom JW, Wallentin L, Connolly SJ, et al. Risk of bleeding with 2 doses of dabigatran compared with warfarin in older and younger patients with atrial fibrillation: an analysis of the randomized evaluation of long-term anticoagulant therapy (RE-LY) trial. *Circulation*. 2011;123(21):2363–2372.
3. Pasi KJ, Rangarajan S, Georgiev P, et al. Targeting of Antithrombin in Hemophilia A or B with RNAi Therapy. *N Engl J Med*. 2017;377(9):819–828.
4. Chan KE, Giugliano RP, Patel MR, et al. Nonvitamin K Anticoagulant Agents in Patients With Advanced Chronic Kidney Disease or on Dialysis With AF. *Journal of the American College of Cardiology*. 2016;67(24):2888–2899.
5. Dobrowolski C, Clark EG, Sood MM. Venous thromboembolism in chronic kidney disease: epidemiology, the role of proteinuria, CKD severity and therapeutics. *J. Thromb. Thrombolysis*. 2017;43(2):241–247.
6. Burnett AE, Mahan CE, Vazquez SR, et al. Guidance for the practical management of the direct oral anticoagulants (DOACs) in VTE treatment. *J. Thromb. Thrombolysis*. 2016;41(1):206–232.
7. Di Minno A, Frigerio B, Spadarella G, et al. Old and new oral anticoagulants: Food, herbal medicines and drug interactions. *Blood Rev.* 2017;31(4):193–203.
8. Fredenburgh JC, Gross PL, Weitz JI. Emerging anticoagulant strategies. *Blood*. 2017;129(2):147–154.
9. Gailani D. Future prospects for contact factors as therapeutic targets. *Hematology Am Soc Hematol Educ Program*. 2014;2014(1):52–59.
10. van Montfoort ML, Meijers JCM. Recent insights into the role of the contact pathway in thrombo-inflammatory disorders. *Hematology Am Soc Hematol Educ Program*. 2014;2014(1):60–65.
11. Younis HS, Crosby J, Huh J-I, et al. Antisense inhibition of coagulation factor XI prolongs APTT without increased bleeding risk in cynomolgus monkeys. *Blood*. 2012;119(10):2401–2408.
12. Löwenberg EC, Meijers JCM, Monia BP, Levi M. Coagulation factor XI as a novel target for antithrombotic treatment. *J Thromb Haemost*. 2010;8(11):2349–2357.
13. Furie B, Furie BC. In vivo thrombus formation. *J Thromb Haemost*. 2007;5 Suppl 1(s1):12–17.
14. Gailani D, Renné T. The intrinsic pathway of coagulation: a target for treating thromboembolic disease? *J Thromb Haemost*. 2007;5(6):1106–1112.
15. Nourse J, Braun J, Lackner K, Hüttelmaier S, Danckwardt S. Large-scale identification of functional microRNA targeting reveals cooperative regulation of the hemostatic system. *J Thromb Haemost*. 2018;43(Suppl 1):854.
16. Vossen CY, van Hylckama Vlieg A, Teruel-Montoya R, et al. Identification of coagulation gene 3'UTR variants that are potentially regulated by microRNAs. *Br J Haematol*. 2017.
17. Sennblad B, Basu S, Mazur J, et al. Genome-wide association study with additional genetic and post-transcriptional analyses reveals novel regulators of plasma factor XI

levels. *Human Molecular Genetics*. 2017.

18. Salloum-Asfar S, Teruel-Montoya R, Arroyo AB, et al. Regulation of Coagulation Factor XI Expression by MicroRNAs in the Human Liver. *PLoS ONE*. 2014;9(11):e111713.

19. Yang DT, Flanders MM, Kim H, Rodgers GM. Elevated factor XI activity levels are associated with an increased odds ratio for cerebrovascular events. *Am. J. Clin. Pathol.* 2006;126(3):411–415.

20. Siegerink B, Rosendaal FR, Algra A. Antigen levels of coagulation factor XII, coagulation factor XI and prekallikrein, and the risk of myocardial infarction and ischemic stroke in young women. *J Thromb Haemost*. 2014;12(5):606–613.

21. Folsom AR, Tang W, Roetker NS, et al. Prospective study of circulating factor XI and incident venous thromboembolism: The Longitudinal Investigation of Thromboembolism Etiology (LITE). *Am. J. Hematol.* 2015;90(11):1047–1051.

22. Salomon O, Steinberg DM, Koren-Morag N, Tanne D, Seligsohn U. Reduced incidence of ischemic stroke in patients with severe factor XI deficiency. *Blood*. 2008;111(8):4113–4117.

23. Al-Horani RA, Afosah DK. Recent advances in the discovery and development of factor XI/XIa inhibitors. *Med Res Rev*. 2018;38(6):1974–2023.

24. Danckwardt S, Hentze MW, Kulozik AE. 3' end mRNA processing: molecular mechanisms and implications for health and disease. *EMBO J.* 2008;27(3):482–498.

25. Lu Y-C, Chang S-H, Hafner M, et al. ELAVL1 modulates transcriptome-wide miRNA binding in murine macrophages. *Cell Rep*. 2014;9(6):2330–2343.

26. Chang S-H, Lu Y-C, Li X, et al. Antagonistic function of the RNA-binding protein HuR and miR-200b in post-transcriptional regulation of vascular endothelial growth factor-A expression and angiogenesis. *J. Biol. Chem.* 2013;288(7):4908–4921.

27. Danckwardt S, Gantzert A-S, Macher-Goeppinger S, et al. p38 MAPK Controls Prothrombin Expression by Regulated RNA 3. *Molecular Cell*. 2011;41(3):298–310.

28. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov*. 2017;16(3):203–222.

29. Thermann R, Hentze MW. Drosophila miR2 induces pseudo-polysomes and inhibits translation initiation. *Nature*. 2007;447(7146):875–878.

30. Iwakawa H-O, Tomari Y. The Functions of MicroRNAs: mRNA Decay and Translational Repression. *Trends Cell Biol.* 2015;25(11):651–665.

Figure 1

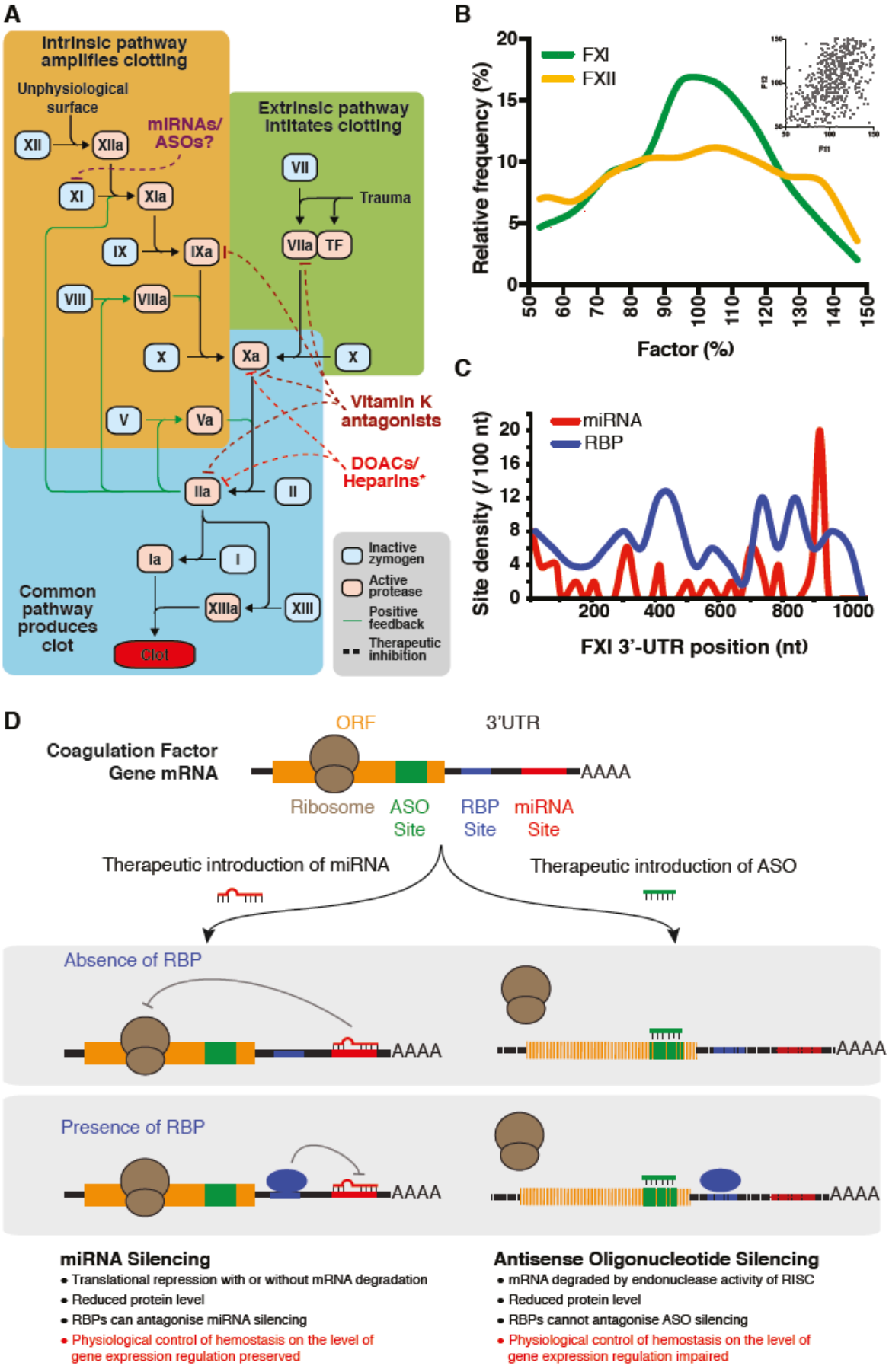
A
Intrinsic pathway amplifies clotting
Unphysiological surface
XII
XIIa
miRNAs/ ASOs?
XI
XIa
IX
IXa
VIII
VIIIa
X
Xa
V
Va
Extrinsic pathway initiates clotting
VII
Trauma
VIIa
TF
X
Vitamin K antagonists
DOACs/ Heparins*
IIa
II
Ia
I
XIIIa
XIII
Common pathway produces clot
Clot
Inactive zymogen
Active protease
Positive feedback
Therapeutic inhibition
B
FXI
FXII
Relative frequency (%)
Factor (%)
C
miRNA
RBP
Site density (/ 100 nt)
FXI 3'-UTR position (nt)
D
Coagulation Factor Gene mRNA
ORF
3'UTR
AAAA
Ribosome
ASO Site
RBP Site
miRNA Site
Therapeutic introduction of miRNA
Therapeutic introduction of ASO
Absence of RBP
Presence of RBP
miRNA Silencing
• Translational repression with or without mRNA degradation
• Reduced protein level
• RBPs can antagonise miRNA silencing
• Physiological control of hemostasis on the level of gene expression regulation preserved
Antisense Oligonucleotide Silencing
• mRNA degraded by endonuclease activity of RISC
• Reduced protein level
• RBPs cannot antagonise ASO silencing
• Physiological control of hemostasis on the level of gene expression regulation impaired

Figure 1. New rational therapeutic targets in the hemostatic system. (A) The hemostatic system is tightly balanced to reduce blood loss by formation of blood clots (hemostasis), while at the same time preventing pathological clot formation (thrombosis). Hemostasis consists of two initial pathways leading to clot formation, the intrinsic (known as contact activation) pathway, and the extrinsic (known as tissue factor) pathway. Exposure of blood to tissue factor (TF) initiates the extrinsic pathway, ultimately leading to clot formation. Under physiological conditions the intrinsic pathway amplifies factor Xa generation rather than initiating hemostasis. Deficiencies of the intrinsic pathway factors XII and XI are usually not associated with abnormal hemostasis but appear to prevent thrombus formation, qualifying them as attractive therapeutic targets. miRNA-mediated control of gene expression provides an attractive avenue for therapeutic targeting of factor XI as opposed to current therapies which target the extrinsic and common pathways (such as Vitamin K antagonists, direct oral anticoagulants (DOACS) or antithrombin dependent* (low molecular weight) heparins). (B) FXI plasma levels exhibit a narrower range as compared to FXII (levels of FXI and FXII from patients admitted to the University Medical Center from 2012 to 2018; values were limited to patients for which both FXI and FXII assays were performed and extremes (levels ≥150% and ≤50%) have been excluded). (C) Density of potential sites for miRNA and RNA-binding proteins (RBPs) across FXI 3'UTR. 125 FXI-3'UTR/miRNA interactions were identified by miTRAP/RNA-seq[15] and of these 41 are mapped to the FXI 3'UTR using miRWalk target site prediction (score >0.5, resulted in 715 total predictions). 392 FXI-3'UTR/RBP interactions identified by miTRAP/MS (unpublished) and of these 66 could be mapped to the FXI 3'UTR using RBPDB target site prediction (score >4, length >4, rel score >0.6, resulted in 4626 total predications). Site density calculated by number of sites present in 50 nt windows over length of the FXI-3'UTR. (D) miRNA-mediated therapeutic targeting may preserve cell intrinsic regulatory mechanisms. Protein expression levels depend not only on the rate of transcription, but on other regulatory mechanisms, including mRNA processing, stability, transport and translational regulation. These post-transcriptional mechanisms have been implicated in the control of key molecular pathways and are primarily mediated by the binding of miRNAs and RNA-binding proteins (RBPs) to regulatory elements of the UTRs of mRNAs, including key components of the blood coagulation system[27]. As opposed to antisense oligonucleotide (ASO) silencing, which inevitably results in degradation of the target mRNA, the partial base-pairing of miRNAs prevents the cleavage activity of RISC with silencing resulting from translational repression[29] and in some cases deadenylation, decapping and finally mRNA degradation[30] (and refs. therein). Significantly miRNA sites in proximity to RBP sites are subject to other mechanisms of physiological control[24-26].