Editorial

Gene therapy-based prophylaxis to rescue the “prima ballerina”

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The activation of factor VII (FVII) represents a key step for the initiation of blood coagulation, amplified by the activation of several serine proteases in the cascade (1). The physiological activity of activated FVII (FVIIa), and thus blood clotting, is triggered when plasma comes into contact with wound-exposed tissue factor (TF) thus forming the TF-FVIIa complex (2), which requires phospholipid membranes because TF is an integral membrane glycoprotein and FVIIa binds to membrane surfaces via its vitamin K-dependent domain (3,4). The TF-FVIIa pathway, defined as the “prima ballerina” of the whole coagulation process (5), includes the activation of factor IX (FIX) and factor X (FX) by limited proteolysis, ultimately leading to fibrin deposition and clot formation. Alterations of the TF-FVIIa pathway, increasing or decreasing its function, play multiple roles in human diseases (*Figure 1*).

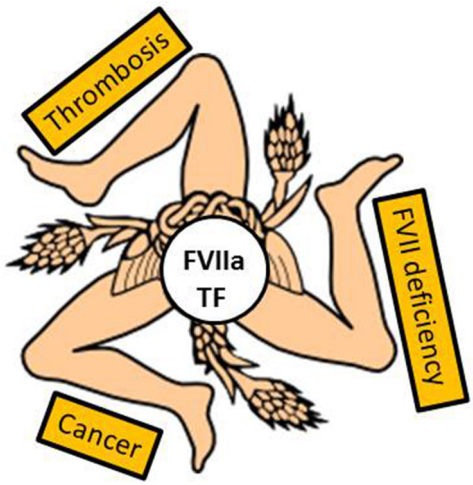

Figure 1 Alterations of the coagulation factor VII (FVII)-tissue factor (TF) pathway are involved in multiple human diseases.

Factor VII (FVII), tissue factor (TF) and cancer

Very interestingly, FVII, TF and the TF-FVIIa complex participate in the pathogenesis of cancer. Both the membrane-bound full-length TF and soluble alternatively spliced TF isoforms have been shown to affect cancer pathophysiology by acting in tumor-associated angiogenesis, tumor growth and metastasis. The TF-FVIIa complex in blood plasma exudate has been shown to stimulate many aspects of cancer progression, including protease-activated receptors, cell growth and motility.

Defined oncogenic transformations drive TF expression in cancer via hypoxia-induced signaling, EGFR- and PTEN-dependent pathways as well as Src-signaling pathways (6). Moreover, various cancer cells ectopically synthesize FVII, which could result in activation of cell motility and invasion. FVII ectopic expression (7) does not depend on transcription factors essential for the physiological hepatic expression, like HNF-4, but still requires Sp1 binding. Histone acetyltransferases p300 and CBP have been found to be recruited to the promoter region of the FVII gene in breast cancer cells. Last, tissue factor pathway inhibitor (TFPI), the primary physiological regulator of FVII-TF-induced blood coagulation, is also expressed in many cancer cells, including breast cancer cells, where it could possess anti-cancer properties. Since the TF-FVIIa complex also modulates thrombogenicity of cancer cells through its procoagulant role, it establishes tight and

multiple links between coagulation and cancer.

Not surprisingly, targeting of the TF-FVIIa pathway offers novel therapeutic options for cancer treatment, and strategies to inhibit ectopic FVII-induced tumor progression without impairment of the physiological hemostatic process are of great interest.

Factor VII (FVII)-tissue factor (TF) and thrombosis

Depending on its pivotal position in the clotting cascade, increased plasma levels of FVII have been deeply investigated in several epidemiological studies as determinants of hypercoagulability. High plasma levels of FVII have been indeed suggested to be predictors of death due to coronary artery disease. *FVII* gene (*F7*) polymorphisms contribute to ample variations in FVII levels in the population and may be associated with the risk of myocardial infarction, which is precipitated by thrombosis. Factor VII genotypes predicting lower FVII levels may have a role in protection against myocardial infarction, despite the presence of severe coronary atherosclerosis (8).

Factor VII (FVII) deficiency

As a deficiency specifically modelling the impaired initiation of coagulation, the congenital defect of FVII (9,10), a rare autosomal recessive disorder (1 in 500,000 people) caused by mutations that affect the plasma levels and/or activity of FVII, has received noticeable attention. Indeed FVII deficiency, despite its modest frequency, is one of the most extensively investigated bleeding disorders. The first mutations in FVII deficiency have been described 25 years ago in Italian patients (11) and registers (12) and database (13) reporting FVII genotyping in more than 1,000 subjects are now available. The clinical symptomatology of FVII deficiency does not completely overlap with that of other more frequent conditions, as Hemophilia A and Hemophilia B. In addition to joint bleeding and hemarthrosis, frequent symptoms in hemophilia, very low FVII levels are associated with gastrointestinal and central nervous system (CNS) bleeds. Whereas CNS bleeds, a life-threatening symptom with devastating post-hemorrhage consequences, affect very severe patients in the first days or weeks after birth, the complete absence of FVII, is considered not compatible with life. Differently from FVIII and FIX deficiencies, ample homozygous deletions of FVII gene have never been described and FVII knock-out mouse models display perinatal lethality. Tiny amount of FVII, maybe lower than that of other clotting factors, seems to be able to confer a basic hemostatic competency and to produce a noticeable amount of thrombin in plasma system. The standard definition of severe deficiency of clotting factors (≤1% coagulant activity in plasma) includes FVII deficient patients with ample differences in bleeding symptoms (from life-threatening to moderate). On the other hand, the majority of patients with FVII deficiency are affected by less severe symptoms such as muscle hematomas and menorrhagia, frequent in a autosomal disorder, and by epistaxis.

Currently, acute bleeding episodes in FVII deficiency are treated by infusion of fresh-frozen plasma, plasma-derived FVII concentrates, prothrombin complex concentrates, and low-dose recombinant activated human FVII (rhFVIIa). Early prophylactic treatment is recommended in young high-risk patients, after the first episode of CNS/gastrointestinal bleeding. These patients have a clear benefit from long-term infusion of FVII or rhFVIIa, and could experience a substantial improvement from stable expression of therapeutic levels of FVII protein produced by gene therapy.

Hemophilia B gene therapy

FIX deficiency/hemophilia B (HB), for the relatively high frequency of an X-linked disease, and for the small size of the gene and encoded protein-similar to those of FVII-, has been the archetypal coagulation disorder for gene therapy. The substantial advances in HB gene therapy constitute the foundations of the more recent advances in other coagulation deficiencies, all favored by adeno-associated viral (AAV) vectors, which are very efficient tools to deliver the transgene encoding the functional clotting factor. A recombinant serotype 8-AAV-vector directed to the liver of severe HB patients resulted in stable and multiyear expression of human FIX, and in a significant reduction of bleeding episodes and use of prophylactic FIX protein (14). The transient increase in liver enzymes observed in most of the patients, resolved with a short course of prednisolone, pointed out a critical issue, the upper limit of AAV8 dosing compatible with human gene therapy using this vector.

Factor VII (FVII) deficiency gene therapy

As compared with FIX, the shorter half-life of FVII (2–3 hours) requires much more frequent infusions than in HB, which makes gene-based therapeutic approaches

particularly attractive for curing FVII deficiency. Previous work by administration in mice of AAV8 encoding mouse FVII produced a stable and efficient transgene expression (15). Differently, in adult nonhuman primates the expression of a human FVII transgene reached a plateau with values <10%. Interestingly, gene transfer by AAV5 in utero of these animals resulted in rapidly declining expression of human FVII after birth, and re-administration by AAV8 in adulthood produced a plateau of approximately 7% normal (15). A splicing mutation-specific approach, based on engineered U1snRNP and able to rescue the correct FVII mRNA, has been proposed in a mouse model (16) expressing a human FVII mutation. These data provided the proof of principle for AAV-mediated gene therapy of FVII deficiency, with noticeable unsolved issues for translation in humans.

Marcos-Contreras and Colleagues have recently reported (17) the successful gene therapy in dogs with an inherited mutation in the FVII gene, able to produce in these animals a severe defect of FVII. This research group in Philadelphia, with a long lasting and outstanding tradition for gene therapy of bleeding disorders, described ten years ago the presence in dogs of the missense mutation Gly96Glu in the FVII gene. This change, which well represents the most frequent mutation type observed in FVII deficient patients, naturally results in very low FVII activity providing a valuable large-animal model of inherited FVII deficiency. Taking advantage of this precious model, in the recent paper they show for the first time that expression of FVII via AAV gene transfer is safe and is also long-term. Importantly, the therapeutic levels have been attained with a single AAV infusion and using vector doses that are considered acceptable in humans. Canine HB studies have enabled the accurate prediction of the AAV vector dose required for gene therapy in humans, and the work published by Marcos-Contreras and colleagues further exemplifies the importance of natural canine models of disease for novel therapeutic approaches.

The use of a species-specific transgene is an additional strength of this study favoring in the coagulation pathway the physiological interactions for the canine FVII protein, first of all canine TF. In these experiments, the hemostatic process is naturally triggered in an animal which is naturally deficient, providing the best model for rescued hemostasis. In addition, this avoids potential species-specific incompatibilities, which would represent major confounding effects for translation in human FVII deficiency.

This study also provides useful information which could rule out complications of substitutive treatment in hemophilias. (I) Paradoxically, thrombotic episodes after substitutive treatment have been described in a relatively high number of patients with FVII deficiency. One of the treated dogs, intentionally exposed to the highest vector and FVII cDNA dose, displayed for years particularly high FVII levels, thus challenging the hemostatic system for potentially prothrombotic conditions. Interestingly, the FVII deficient dog exposed to FVII levels >700% was not affected by thrombotic episodes, which may indicate a remarkably wide therapeutic window. This observation supports a noticeable safety of this gene therapy-based prophylaxis, even after exposure to very high FVII levels, more than one order of magnitude larger than the therapeutic threshold; (II) a major complication of hemophilia treatment consists in high titer and inhibitory antibodies directed to the proteins (FVIII, FIX) infused for therapeutic purposes. Gene deletions causing completely null conditions favor the onset of antibodies both in HB and HA whereas inhibitor formation is less frequent for missense mutations, such as the canine Gly96Glu substitution. Only the dog exposed to high levels of FVII transgene expression displayed an IgG2 response to FVII, which may resemble the development of inhibitors. However, it was transient and did not inhibit the FVII expressed by the transgene. Moreover, none of the dogs with a physiological level of transgene expression exhibited an inhibitory immune response, which is promising for gene-based prophylaxis in human FVII deficiency.

The gene therapy approach reported by Marcos-Contreras and Colleagues provides in a large-animal model novel data on the longevity and stability of therapeutic levels of FVII expression. Moreover, these experiments address safety issues in terms of absence of immunologic and thrombotic complications, and of physiological expression site and levels of the transgene. Translation of this approach has the potential for long-term and stable expression of curing levels of FVII protein in human plasma, thus avoiding the daily protein infusions and substantially improving the quality of life (QoL) of severe FVII deficient patients.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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