ADAMTS13: Molecular biology and clinical implications

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Abstract: Objectives: Aim of this work to illustrate the molecular biology and pathobiology of ADAMTS13 and its therapeutic implications. **Data Sources:** Medline databases (PubMed, Med scape, Science Direct.) **Article Selection:** The article studied the molecular biology of ADAMTS13 and its role in diseases and therapy. if the articles did not fulfill the inclusion criteria they were excluded. **Data Synthesis:** Short reviews were made on ADAMTS13 structure and its role in different diseases and therapy. **Conclusion:** The knowledge of some mechanistic aspects of ADAMTS13 catalysis and its regulation, the development of sensitive and reliable assays in the clinical diagnostics of TMAs and the nature of modifiers of ADAMTS13 activity on VWF multimers in patients affected by TMAs.
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1. Introduction

ADAMTS13 (adisintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) also known as von Willebrand factor-cleaving protease (VWFCP) is a zinc-containing metalloproteaseenzyme that cleaves von Willebrand factor (vWf), a large protein involved in blood clotting. It is secreted in blood and degrades large vWfmultimers, decreasing their activity **[1].**

vWF is a multimeric plasma glycoprotein that have very important role in platelet adhesion and aggregation on vascular lesions., is synthesized by vascular endothelial cells and is present in human plasma at concentrations of 10 μg/mL**[2].**

ADAMTS13 gene encodes a predicted 1,427-amino acid protein. The ADAMTS13 protein has a signal peptide, followed by a short propeptide domain ending in a potential propeptide convertase cleavage site at amino acids 71 to 74 (RQRR), suggesting that proteolytic processing, either in the trans Golgi or the cell surface, is required for activation **[3].**

vonWillebr and factor-cleaving protease (ADAMTS13) activity (< 5% that in normal plasma) has been detected in most patients with a diagnosis of thrombotic thrombocytopenic purpura (TTP) but not observed in those with a diagnosis of hemolytic uremic syndrome. However, ADAMTS13 deficiency has been not to be specific for TTP, since it was seen in various thrombocytopenic disorders as severe sepsis or septic shock, heparin-induced thrombocytopenia, idiopathic thrombocytopenic purpura, sever malaria or other hematologic, or miscellaneous conditions**[4].**

2. Materials and Methods

Search Strategy:

We reviewed papers on the influence of complement dysregulation on blood diseases from Medline databases which are (Pub Med, Medscape, Science Direct) and also materials available in the Internet. We used ADAMTS13/Molcularbiology/ Synthesis/ Structure/ Thrombotic thrombocytopenic purpura / atypical hemolytic uremic syndrome/ Disseminated Intravascular Coagulation / Immune Thrombocytopenic Purpura/ Recombinant ADAMTS13 / Eculizumab as searching terms.

Article Selection:

All the reviews were independently assessed for inclusion. They were included if they fulfilled the following criteria:

Inclusion criteria of the published studies:

-Published in English language.

-Focused on ADAMTS13Molcular biology and its role in diseases and therapy.

-Discussed the relation between ADAMTS13 Molcular biology and its role in diseases and therapy.

Data Synthesis:

A structured systematic review was performed illustrated with diagrams.

3. Results:

The initial search presented 260 articles, papers and journals about the title of the article with the key words mentioned; extraction was made, including assessment of quality and validity of papers. 110

published articles met the inclusion criteria. Studies concerning with complement dysregulation in blood disorders was collected; each study was reviewed independently; obtained data is rebuilt in new language and arranged in topics through the article.

4. Discussion

A**d**isintegrin **a**nd **m**etalloproteinase with a **t**hrombo**s**pond in type 1 motif, member **13** (ADAMTS13) also known as von Willebrand factor-cleaving protease(VWFCP) is zinc-containing metalloprotease enzyme that split, von Willebrand factor (vWf), a large protein involved in blood clotting. It is secreted in blood and degrades large vWf multimers, decreasing their activity. vWF is secreted from endothelial cells by the regulated pathway as a very large (20_103 kDa) polymer of the mature 220-kDa polypeptide**[1].**

ADAMTS13 is primarily synthesised in the liver of humans, mice, and rats The mRNA encoding the full-length ADAMTS13 (~4.3 kb) is detected only in the liver by Northern blotting analysis. However, a truncated form of ADAMTS13 mRNA (~2.4 kb) is found in other tissues such as placenta and skeletal muscle by the same method Using reverse polymerase chain reaction (PCR), fragments of ADAMTS13 mRNA are amplified in many tissues including the kidneys, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes**[5].**

The ADAMTS enzymes are secreted, multi-domain matrix-associated zinc metalloendopeptidases that have diverse roles in tissue morphogenesis and patho-physiological remodeling, in inflammation and in vascular biology. The human family includes 19 members that can be sub-grouped on the basis of their known substrates, namely the aggrecanases or proteoglycanases (ADAMTS1, 4, 5, 8, 9, 15 and 20), the procollagen N-propeptidases (ADAMTS2, 3 and 14), the cartilage oligomeric matrix protein-cleaving enzymes (ADAMTS7 and 12), the von-Willebrand Factor proteinase (ADAMTS13) and a group of orphan enzymes (ADAMTS6, 10, 16, 17, 18 and 19) **[6].**

Control of the structure and function of the extracellular matrix (ECM) is a central theme of the biology of the ADAMTS, as exemplified by the actions of the procollagen-N-propeptidases in collagen fibril assembly and of the aggrecanases in the cleavage or modification of ECM proteoglycans. Defects in certain family members give rise to inherited genetic disorders, while the aberrant expression or function of others is associated with arthritis, cancer and cardiovascular disease **[6].**

ADAMTS13 consists of numerous domains including a metalloprotease domain, a disintegrin domain, several thrombospondin type 1 (TSP1) repeats, a cysteine-rich domain, a spacer domain and 2 CUB (Complement c1r/c1s, sea Urchin epidermal growth factor, and Bone morphogenetic protein) domains. ADAMTS13 cleaves a single peptide bond (Tyr1605-Met1606) in the central A2 domain of the VWF molecule **[7].**

The ADAMTS13 gene provides instructions for making an enzyme that is involved in blood clotting. After an injury, clots normally protect the body by sealing off damaged blood vessels and preventing further blood loss. The ADAMTS13 enzyme processes a large protein called von Willebrand factor, which also plays a role in clot formation. The unprocessed form of von Willebrand factor interacts easily with cell fragments called platelets, which circulate in the bloodstream and are essential for blood clotting. The factor helps platelets stick together and adhere to the walls of blood vessels, forming temporary clots. The ADAMTS13 enzyme cuts von Willebrand factor into smaller pieces. By processing von Willebrand factor in this way, the enzyme prevents it from triggering the formation of unnecessary blood clots **[8].**

Recently, an animal study demonstrates that ADAMTS13 may have thrombolytic activity by dissolvinga preformed clot induced by FeCl3injury in venules. Such thrombolytic activity cannot bead equately explained by the cleavage of VWF alone. South et al reported that an ADAMTS13 variant lacking the C-terminal TSP1 2-8 repeats and CUB domains (S) or a gain-of-function ADAMTS13 variant (GoF), was able to cleave fibrinogen directly. Such an activity was also observed with full-length ADAMTS13 (FL) after being bound by the VWF-D4 domain **[9].**

Autoantibody epitope mapping has not only further characterized the mechanism of the disease, but also uncovered the structure–function relationship of ADAMTS13. Most acquired TTP patients have multiple anti-ADAMTS13 antibodies that bind to several domains Binding epitopes, including residues Glu376, Gln159–Asp166, Tyr 305–Glu327, and Asn308 in the metalloprotease and disintegrin domains have been identified **[10].**

However, the most frequent antibody epitope region is found within the cysteine-rich and spacer domain, particularly the spacer domain. In the mapping of the antigenic regions on ADAMTS13, the most common characteristic is the involvement of five loops located on the spacer domain **[11].**

Von Willebrand factor (vWF) is a blood glycoprotein involved in hemostasis. It is deficient or defective in von Willebrand disease and is involved in a large number of other diseases, including thrombotic thrombocytopenic purpura, Heyde's syndrome, and

possibly hemolytic-uremic syndrome. Increased plasma levels in a large number of cardiovascular, neoplastic, and connective tissue diseases are presumed to arise from adverse changes to the endothelium, and may contribute to an increased risk of thrombosis **[12].**

Von Willebrand factor is synthesized in endothelial cells and megakaryocytes as a primary translation product of 2813 amino acids; it subsequently undergoes considerable processing, including dimerization and multimerization to very large forms. The primary translation product contains a signal peptide of 22 amino acids followed by a propeptide of 741 residues, also known as VWF propeptide (von Willebrand antigen II), and a mature subunit of 2050 amino acids**[13].**

Deficiency of ADAMTS13 was originally discovered in Upshaw Schulman Syndrome. By that time it was already suspected that TTP occurred in the autoimmune form as well, owing to its response to plasmapheresis and characterisation of IgG inhibitors. Since the discovery of ADAMTS13, specific epitopes on its surface have been shown to be the target of inhibitory antibodies**[1]**.

However, ADAMTS13 deficiency has been claimed not to be specific for TTP, since it was observed in various thrombocytopenic disorders as severe sepsis or septic shock, heparin-induced thrombocytopenia, idiopathic thrombocytopenic purpura, or other hematologic, or miscellaneous conditions**[4].**

Thrombotic thrombocytopenic purpura:

Thrombotic thrombocytopenic purpura (TTP) is a rare clinical syndrome characterized by the formation of disseminated platelet aggregates in small blood vessels and consequent microangiopathic haemolytic anaemia. The aggregated platelets can cause ischaemia, hypoxia and abnormal function of the affected organs. The classical clinical pentad of TTP consists of microangiopathic haemolytic anaemia, thrombocytopenia, fluctuating neurological signs, impaired renal function and fever **[14].**

A hereditary form of TTP is called the Upshaw-Schulman syndrome; this is generally due to inherited deficiency of ADAMTS13 (frameshift and point mutations). Patients with this inherited ADAMTS13 deficiency have a surprisingly mild phenotype, but develop TTP in clinical situations with increased von Willebrand factor levels, e.g. infection. Reportedly, less than 1% of all TTP cases are due to Upshaw-Schülman syndrome. Patients with Upshaw-Schulman syndrome have 5–10% of normal ADAMTS-13 activity **[15].**

Acute idiopathic TTP is the most common form of TTP. It is an autoimmune disease characterized by antibodies, usually IgG, directed against ADAMTS13. The incidence is four to six cases per million of the population per year in the United States and six cases per million per year in the UK **[16].**

Thrombotic thrombocytopenic purpura was originally characterized by a pentad of thrombocytopenia, MAHA, fluctuating neurological signs, renal impairment and fever, often with insidious onset. However, TTP can present without the full pentad; up to 35% of patients do not have neurological signs at presentation and renal abnormalities and fever are not prominent features. The revised diagnostic criteria state that TTP must be considered in the presence of thrombocytopenia and MAHA alone. This can result in an increased referral of other TMAs **[17].**

Therapy should be initiated if the diagnosis of thrombotic thrombocytopenic purpura (TTP) is seriously considered **[18].**

Plasmapheresis should be administered within 24 hours of the clinical onset of TTP, and it should be administered daily for 3 consecutive days; during plasmapheresis, 1-1.5 of the plasma volume should be exchanged. In patients who present with neurological deficit and signs of cardiac ischaemia**[19].**

Two plasmapheresis treatments per day are necessary during the first three days of the disease. Daily plasmapheresis should be continued two days after the platelet count gets higher than 150x10\ l, and then it should be stopped **[20].**

Corticosteroids are commonly given to patients with TTP. Responses to corticosteroid therapy alone have been documented. Increasing evidence supports the use of the anti-CD20 monoclonal antibody rituximab in cases of TTP refractory to plasma exchange, with resolution of acute disease and prolonged remission**[21].**

British guidelines recommend offering rituximab to patients with refractory or relapsing immune-mediated TTP, and considering rituximab as part of first-line therapy, along with plasma exchange and steroids, in acute idiopathic TTP with neurological/cardiac pathology, as those cases are associated with high mortality**[18].**

Caplacizumab, an anti–von Willebrand factor humanized single-variable-domain immunoglobulin, may prevent microthrombus formation by blocking the binding of platelets to ultralarge von Willebrand factor multimers. In a randomized phase 2 study in 76 patients with acute acquired TTP, the addition of caplacizumab to stand-of-care treatment led to faster normalization of the platelet count than did placebo **[22].**

Atypical Hemolytic Uremic Syndrome:

The disease affects both children and adults and is characterized by systemic thrombotic microangiopathy (TMA), the formation of blood clots in small blood vessels throughout the body, which can lead to stroke, heart attack, kidney failure, and death **[23].**

The complement system activation may be due to mutations in the complement regulatory proteins (factor H, factor I, or membrane cofactor protein), or is occasionally due to acquired neutralizing autoantibody inhibitors of these complement system components, for example anti–factor H antibodies In healthy individuals, complement is used to attack foreign substances. However, in most patients with aHUS, it has been demonstrated that chronic, uncontrolled, and excessive activation of complement can result from production of anti-factor H autoantibodies or from genetic mutations in any of several complement regulatory proteins (e.g., factor H, factor HR1 or HR3, membrane cofactor protein, factor I, factor B, complement C3, and thrombomodulin) **[24].**

This results in platelet activation, damage to endothelial cells, and white blood cell activation, leading to systemic TMA, which manifests as decreased platelet count, hemolysis, damage to multiple organs, and often, death **[25].**

Feng et al. measured complement activity and ADAMTS13 function, and completed mutation screening of multiple complement genes and ADAMTS13 in a large cohort of aHUS patients. And they implicate ADAMTS13 in the pathogenesis of aHUS and suggest that there exists a subgroup of patients with aHUS in whom complement is dysregulated and ADAMTS13 function is reduced **[26].**

Before the introduction of Before the introduction of eculizumab, a monoclonal antibody that is a first-in-class terminal complement inhibitor, management options for patients with aHUS were extremely limited. Guidelines issued by the European Paediatric Study Group for HUS recommend rapid administration of plasma exchange or plasma infusion (PE/PI), intensively administered daily for 5 days and then with reducing frequency **[27].**

Disseminated Intravascular Coagulation

Disseminated intravascular coagulation (DIC), is a pathological process characterized by the widespread activation of the clotting cascade that results in the formation of blood clots in the small blood vessels throughout the body. This leads to compromise of tissue blood flow and can ultimately lead to multiple organ damage. In addition, as the coagulation process consumes clotting factors and platelets, normal clotting is disrupted and severe bleeding can occur from various sites**[28].**

Lower molecular weight forms of ADAMTS13 were found in the plasma of patients with sepsis-induced DIC, suggesting that the deficiency of ADAMTS13 was partially caused by its cleavage by proteases in addition to decreased synthesis in the liver. These data suggested that severe secondary ADAMTS13 deficiency can be associated with sepsis-induced DIC and may contribute to the development of renal failure **[29].**

Immune Thrombocytopenic Purpura

Immune thrombocytopenia (ITP) is defined as isolated low platelet count (thrombocytopenia) with normal bone marrow and the absence of other causes of thrombocytopenia. It causes a characteristic purpuric rash and an increased tendency to bleed. Two distinct clinical syndromes manifest as an acute condition in children and a chronic condition in adults. The acute form often follows an infection and has a spontaneous resolution within two months **[30].**

Charlotte et al. were analyzed 93 samples were taken from 48 patients with ITP for VWF:Ag levels. Plasma concentrations of ADAMTS13 and IL-6 were analyzed in 58 and 34 plasma samples respectively. Plasma VWF antigen (VWF:Ag) levels were elevated in patients with ITP compared to control subjects. Levels of ADAMTS13 in patients with ITP were lower than those for control subjects. They concluded that: increased ratio of VWF: ADAMTS13 may be contributory in the pathogenesis of thrombosis, in particular in patients with acute and unstable disease**[31].**

Role of ADAMTS13 in liver diseases

Decreased ADAMTS13 activity may be involved not only in sinusoidal microcirculatory disturbances, but also subsequent progression of liver injuries, eventually leading to multiorgan failure. This concept can be applied to the development or aggravation of liver diseases, including liver cirrhosis, alcoholic hepatitis, veno-occlusive disease, and adverse events after liver transplantation **[32].**

Emerging evidence supports the concept of a rebalanced hemostatic state in liver disease as a result of a commensurate decline in pro hemostatic and anti hemostatic drivers. Hugenholtz et al assessed levels and functionality of the platelet-adhesive protein von Willebrand factor (VWF) and its cleaving protease ADAMTS13 in the plasma of patients with acute liver injury and acute liver failure (ALI/ALF) **[33].**

Platelet adhesion and aggregation were better supported by plasma of patients with ALI/ALF when compared with control plasma. Low ADAMTS13 activity, but not high VWF antigen, was associated

with poor outcome in patients with ALI/ALF as evidenced by higher grades of encephalopathy, higher transplantation rates, and lower survival. VWF or ADAMTS13 levels were not associated with bleeding or thrombotic complications **[33].**

Recombinant ADAMTS13 and the treatment of acquired TTP:

Animal models have demonstrated the protective effects of recombinant human ADAMTS13 (rADAMTS13). Schiviz *et al*. reported that the prophylactic administration of rADAMTS13 in ADAMTS13 knockout mice that were challenged with human VWF containing ultra-large VWF multimers. Untreated animals rapidly showed symptoms and developed a schistocytosis, severe thrombocytopenia, increased lactate dehydrogenase, and extensive platelet aggregation and myocardial necrosis**.** The prophylactic administration of rADAMTS13 in this mouse model protected mice from the development of TTP. While this approach is seemingly going to be more applicable to congenital TTP, there are in vitro data to suggest that rADAMTS13 may be able to overcome the inhibitor present and restore ADAMTS13 protease function**[34].**

Plaimauer et al. used plasma samples from acquired TTP patients that were adjusted to predefined inhibitor titers to study the ability of rADAMTS13 to restore ADAMTS13 protease function and demonstrated a linear relationship between the inhibitor titer measured and the effective rADAMTS13 concentration needed to restore ADAMTS13 protease function. This potential approach to therapy using rADAMTS13 is promising with clinical trials in patients with congenital TTP planned to begin soon**[35].**

Recombinant ADAMTS13 variants and acquired TTP:

Another unique and ingenious approach to the treatment of acquired TTP originates from an increased understanding of the specific antibody binding sites of anti-ADAMTS13 protease, Autoantibodies targeting the ADAMTS13 protease in patients with acquired TTP commonly bind to the spacer domain of ADAMTS13, a region that is critical for the recognition and proteolysis of VWF. Jian and colleagues hypothesized that an alteration of an exosite in the spacer domain of ADAMTS13 could generate variants, resulting in a decreased autoantibody binding, but with preserved ADAMTS13 protease function. Indeed, two mutant ADAMTS13 variants were generated that demonstrated increased specific activity against a VWF73 substrate, but at the same time were more resistant to anti-ADAMTS13 antibodies from patients with acquired TTP. While still early in the preclinical development, these data suggest that these modified ADAMTS13 variants could potentially be an effective treatment or adjunct to the treatment of acquired TTP **[36].**

Conclusion:

After the discovery that normal plasma contains a zinc protease able to specifically proteolyze VWF, the past decade has witnessed the most exciting advances in the history of studies on the pathogenesis of TMAs.

The knowledge of some mechanistic aspects of ADAMTS13 catalysis and its regulation, the development of sensitive and reliable assays in the clinical diagnostics of TMAs and the nature of modifiers of ADAMTS13 activity on VWF multimers in patients affected by TMAs.

From a biotechnological standpoint, industrial production of partially deleted ADAMTS13, non-suppressible by pathological auto-antibodies, may circumvent the difficulties that replacement therapies with recombinant full-length ADAMTS13 may encounter in patients with acquired TTP.

Finally, basic research to clarify the immunological mechanisms of generation of ADAMTS13 inhibitors will aid in the discovery of new strategies able to improve the prevention, diagnosis and management of TMAs.

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