Chapter 15

ADAMTS-13
Thrombotic Thrombocytopenic Purpura

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Abstract: von Willebrand factor (VWF) is a plasma glycoprotein that mediates platelet adhesion and aggregation at the site of vessel injury. Studies on the homeostasis of VWF led to the discovery that it is cleaved in the circulation by a zinc metalloprotease, ADAMTS-13, in a shear stress dependent manner. ADAMTS-13 is phylogenetically most different from other members of the reploysin-type ADAMTS zinc metalloprotease family. In the presence of ADAMTS-13, shear stress on VWF promotes its cleavage by ADAMTS-13 to smaller, less active forms. In the absence of ADAMTS-13, shear stress increases the platelet-aggregating activity of VWF, resulting in accumulation of super-active forms of VWF and subsequently intravascular platelet thrombosis, as observed in patients with thrombotic thrombocytopenic purpura (TTP). In TTP, autoimmune inhibitors of the protease or genetic mutations of the ADAMTS-13 gene cause a severe deficiency of ADAMTS-13 in plasma. Analysis of ADAMTS-13 enables the differentiation of TTP from other types of microvascular thrombosis on a pathogenetic basis and facilitates advances in the diagnosis and therapy of the disease.

Key words: ADAMTS-13, von Willebrand factor, thrombotic thrombocytopenic purpura.

1. INTRODUCTION

von Willebrand factor (VWF) is an endothelial cell-derived glycoprotein that mediates platelet hemostatic plug formation under high shear stress conditions (Ruggeri 2003). Although it exists in the plasma fraction of circulation as a series of multimers, these multimers are not detectable in endothelial cells or their culture media (Tsai et al. 1989; Kaul et al. 1993).
Studies on the genesis of VWF multimers led to the discovery of a protease, ADAMTS-13, whose evidence of existence was previously based on the imprint it left on von Willebrand factor (Zimmerman et al. 1986). Investigations of the homeostasis of von Willebrand factor were instrumental in linking ADAMTS-13 deficiency to a thrombotic disorder, thrombotic thrombocytopenic purpura (TTP).

2. MOLECULAR BIOLOGY OF ADAMTS-13

Human ADAMTS-13 gene contains 29 exons spanning approximately 37 kb on chromosome 9q34 (Levy et al. 2001; Soejima et al. 2001; Zheng et al. 2001). The regulatory elements of the gene have not been defined. Alignment of human and mouse ADAMTS-13 sequences surrounding the transcription initiation site reveals no outstanding region of homology. Neither the human nor mouse putative promoter has a TATA box or a CpG island. Motif analysis has not revealed striking patterns of transcription factor binding sites.

The transcripts of ADAMTS-13 are relatively complex. At least four splicing, and several truncated variants have been described. Because some of these variants profoundly change the structure of the predicted protein, it is speculated that ADAMTS-13 function may vary in different tissues. Multiple-tissue Northern blotting demonstrated that ADAMTS-13 encodes a 4.7-kb transcript primarily in the liver, and a 2.4-kb transcript is detectable in placenta, skeletal muscle, and certain tumor cell lines (Levy et al. 2001; Soejima et al. 2001; Zheng et al. 2001; Cal et al. 2002).

The ADAMTS-13 isoform with VWF cleaving activity is almost certainly the one with all 29 exons, since it has the right molecular weight and cDNA length and since transfection studies of this cDNA demonstrated that this protein has proteolytic activity.

The full-length transcript encodes a precursor polypeptide with 1427 amino acid residues. The amino acid sequence contains 10 consensus sites for N-linked glycosylation, several potential sites for O-linked glycosylation, and one consensus site for C-mannosylation (Zheng et al. 2001). Expression studies revealed that ADAMTS-13 is synthesized in the cells as a 180-kD, instead of the calculated 145-kD, protein, indicating that the protein undergoes extensive glycosylation and other modifications. Smaller forms of ADAMTS-13 (170 kD, 160 kD, and 120 kD) have been isolated from the plasma. These minor forms contain identical N-terminal amino acid sequence and may derive from the shorter transcripts or proteolytic truncation.