1838

BINDING SITE OF VITAMIN K-DEPENDENT PROTEIN S ON C4b-BINDING PROTEIN. K. Suzuki, J. Nishioka, H. Kusumoto and Y. Deyashiki. Department of Laboratory Medicine, Mie University School of Medicine, Tsu City, Mie 514, Japan.

Protein S, a cofactor for activated protein C, reversibly complexes with a regulatory complement component C4b-binding protein (C4bp) in plasma. In plasma of patients with congenital protein S deficiency, most protein S exists as a complex with C4bp, which has no cofactor activity. C4bp (Mw 550,000) is composed of approximately seven subunits with Mw 75,000 which are linked by disulfide bonds near the carboxyl-terminus. We report here about the complex formation between protein S and C4bp particularly on the binding site of protein S on C4bp molecule. Protein S and C4bp were purified from human plasma. Seventeen mouse monoclonal antibodies against C4bp were prepared. Chymotrypsin-digested C4bp was separated on gel filtration into a fragment with Mw 160,000 derived from the carboxyl-terminal core of the intact C4bp and fragments with Mw 48,000 from the amino-terminus. The carboxyl-terminal fragment with Mw 160,000 was found to be composed of approximately seven polypeptides with Mw 25,000, which were linked by disulfide bonds. The experiments using these fragments and the monoclonal antibodies showed that: (1) Protein S bound not only to the intact C4bp, but also to the fragment with Mw 160,000. (2) The fragment with Mw 160,000 inhibited the binding of protein S to C4bp, but the fragment with Mw 48,000 did not. (3) One of the seventeen monoclonal antibodies blocked the inhibition of C4bp on the cofactor activity of protein S. (4) This antibody inhibited C4bp binding to protein S. (5) The antibody bound to the fragment with Mw 160,000. Based on these results, protein S was suggested to lose its cofactor activity for activated protein C by binding to the carboxyl-terminal core of C4bp where seven subunits are linked by disulfide bonds.

1839

INDEPENDENT ISOLATION OF HUMAN PROTEIN S cDNA AND THE ASSIGNMENT OF THE GENE TO CHROMOSOME 3. J.K. Ploos van Amstel (1), A.L. van der Zanden (1), E. Bakker (2), P.H. Reitsma (1), R.M. Bertina (1). Haemostasis and Thrombosis Research Unit, Leiden University Hospital, Leiden, The Netherlands (1), Anthropogenetica, Sylviuslaboratory, Leiden, The Netherlands (2).

Protein S is a vitamin K-dependent glycoprotein, that serves as a cofactor of activated protein C. A hereditary deficiency in protein S is associated with an increased risk for the development of venous thrombosis. The deficiency is inherited as an autosomal dominant trait. We isolated a cDNA coding for protein S and assigned its gene to chromosome 3.

A human liver cDNA library in phage λgt11 (complexity 1.2 x 10^6, D. Stafford, Chapel Hill) was screened by using immunopurified polyclonal anti-protein S IgG as a probe. Approximately 1.5 x 10^6 recombinants of the amplified library were screened. Out of eighteen positive clones one clone was found, after nucleotide sequence analysis, to code for a peptide with a high degree of homology with the carboxy terminal region of the already published bovine protein S. This clone pSP84 (450 bp) was used as a probe to screen a human liver cDNA library in plasmid pUC9. From this library we isolated several positive clones. Clone pSUL5 contained the largest insert (2200 base pairs). Dideoxy sequencing revealed that it codes for 330 amino acids of the carboxy terminal part of protein S. Furthermore, it contained a 1200 base pairs 3' untranslated region. The predicted amino acid sequence did not differ from the published sequence of human protein S, although at the nucleotide level some differences could be detected.

Clone pSUL5 was used to localize the protein S gene to its chromosome. The assignment was done by hybridization to Pst I digested DNA from human-hamster c.q. human-mouse somatic cell hybrids. In this way we got strong indication that the protein S gene is located on chromosome 3.

1840

RESTRICTION ANALYSIS AND SOUTHERN BLOTTING OF TOTAL HUMAN DNA REVEALS THE EXISTENCE OF MORE THAN ONE GENE HOMOLOGOUS WITH PROTEIN S cDNA. J.K. Ploos van Amstel, A.L. van der Zanden, P.H. Reitsma, R.M. Bertina. Haemostasis and Thrombosis Research Unit, Leiden University Hospital, Leiden, The Netherlands

A deficiency in protein S, the cofactor of activated protein C, is associated with an increased risk for the development of venous thrombosis. It is inherited as an autosomal dominant disorder. To improve the detection of heterozygotes in affected families, we have started to search for restriction fragment length polymorphism (RFLP) in the protein S gene. This study revealed the existence of two genes containing sequences homologous to protein S cDNA.

Three non-overlapping fragments of clone pSUL5, which codes for the carboxy-terminal part of protein S and contains the complete 3' untranslated region, were isolated and used as probes in search for RFLP of the protein S gene.

Surprisingly the non-overlapping probes shared more than one hybridizing band. The hybridization took place under stringent assay conditions.

This observation is contradictory to the intron-exon organization of a gene and suggests the existence of two genes, containing sequences homologous with pSUL5. Both genes could be assigned to chromosome 3 by mapping through somatic cell hybrids. Whether two functional protein S genes are present in the human genome remains to be established.

1841

GENE STRUCTURE OF VITAMIN K-DEPENDENT PROTEIN S; A REGION HOMOLOGOUS TO SEX HORMONE BINDING GLOBULIN (SHBG) REPLACES THE SERINE PROTEASE REGION OF FACTORS IX, X AND PROTEIN C. C-M. Edenbrandt, S. Gershagen, P. Fernlund, R. Wydro*, J. Stenflo and Å. Lundwall. Department of Clinical Chemistry, University of Lund, Malmö General Hospital, Malmö, Sweden and *Integrated Genetics, Framingham, MA, USA.

It has recently been shown that the similarity between coagulation factors IX, X and protein C in the protein sequence is also evident in the organization of their genes. To further elucidate the relation of protein S to the other vitamin K-dependent clotting factors, we are now characterizing the human protein S gene. The size of the gene was estimated to be more than 45 kb, by hybridization of a cDNA for human protein S with chromosomal DNA in a Southern blot.We have isolated three overlapping clones from a human genomic DNA library in bacteriophage λ Charon 4A, which cover approximately 40 kb of the gene. The clones have been mapped by single- and double restriction enzyme digestion. Genomic subclones in pUC 18 which hybridize with cDNA probes for protein S have been isolated and sequenced to establish the intron/exon structure of the gene. The 5'- part of the human protein S gene closely resembles the corresponding part of the genes for factors IX, X and protein C. However, the thrombin sensitive region (amino acids 46-75), which is unique for protein S among the vitamin K-dependent clotting factors, is coded for by a separate exon. The 3'- end of the protein S gene, coding for amino acids 247-635, is not homologous to the catalytic region of the vitamin K-dependent serine proteases but shows a significant homology to human sex hormone binding globulin (SHBG).