Localization of the Gene for Coagulation Factor XIII a-Chain to Chromosome 6 and Identification of Sites of Synthesis

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Abstract

Factor XIII, the clotting factor essential for covalent stabilization of the fibrin clot, is a heterodimer consisting of a_2 and b_2 subunits, with catalytic function residing in the a-chain. In order to address questions regarding sites of synthesis and chromosomal localization of the Factor XIII a-chain, cDNA was cloned from a λgt11 human placental cDNA library. Nucleotide and amino acid sequences were determined from the cDNA. Amino acid sequencing of purified platelet Factor XIII a-chains confirmed the authenticity of the λgt11 clone. The gene for Factor XIII a-chain was mapped uniquely to chromosome 6. Northern blot analysis of human placental and U937 (monocytelike) cell poly $(A)^+$ mRNA showed a single ~ 4.0-kb message for the Factor XIII a-chain. These results provide conclusive evidence that the a-chain is synthesized by placenta and monocyte cell lines.

Introduction

Factor XIII, the fibrin-stabilizing factor, is the zymogen for Factor XIIIa, which covalently cross-links fibrin monomers into a stable fibrin clot in the final stages of the blood coagulation cascade. Plasma Factor XIII exists as a tetrameric complex of polypeptides consisting of two a-chains and two b-chains held together by noncovalent bonds. The complex a_2b_2 found in plasma has a molecular weight of ~ 300,000: relative molecular weight (M_r) of each a-chain is ~ 75,000 and each b-chain is ~ 80,000 (1). The catalytic function of plasma Factor XIII resides in the a-chain (2). Plasma Factor XIII is activated to Factor XIIIa by thrombin, which cleaves a 36-residue peptide from the amino terminus of the a-chains (3). Cleavage is followed by dissociation of the b-chains in the presence of calcium (4).

Factor XIIIa, a transglutaminase, catalyzes the formation of intermolecular γ-glutamyl-ε-lysine bridges between side chains of fibrin molecules. The result is an exceedingly intricate and organized clot of high tensile strength (5) with increased resistance to degradation by plasmin (6). Factor XIII crosslinking of fibrin, collagen, actin, and myosin substrates is believed to be important in wound healing, thrombus organization, and clot retraction (7).

Factor XIII is present in high levels in placental and uterine tissues (8, 9). Congenital deficiency of this factor leads uniformly to spontaneous abortion if prophylactic replacement therapy is not maintained throughout pregnancy (10). In addition to gestational loss, congenital Factor XIII deficiency is characterized by umbilical bleeding, hematomas, intracranial bleeding, and delayed postoperative bleeding (11), as well as slow wound healing and abnormal scar formation (12).

Factor XIII a-chains are present in human placenta, platelets, megakaryocytes, uterus (9, 13, 14) and monocytes and macrophages (15, 16). Factor XIII in platelets, placenta, monocytes and macrophages is composed of a-chains only, and these are identical to plasma a-chains by amino acid composition (2) and electrophoretic (2, 15) and immunochemical (8, 15) analyses. a-Chains have been found in human liver (17) and the human hepatoma cell line Hep G2 (18) by some investigators, but not by others (14). While the protein has been identified in monocytes and macrophages, the phagocytic nature of these cells raises the possibility that the a-chains are endocytosed from the plasma rather than synthesized directly.

The chromosomal location of the Factor XIII a-chain gene is also controversial. Board (19) and Olaisen (20) both reported linkage of Factor XIII a-chain to the HLA-region genes on chromosome 6, based on population genetics studies and linkage analyses of a-chain protein alleles with other polymorphic protein markers. Graham (21), on the other hand, did not find significant linkage of the Factor XIII a-chain to two chromosome 6 markers.

With some of these questions in mind, we cloned cDNA for the Factor XIII a-chain and investigated the issues directly, using DNA probes for the first time.

Methods

Isolation of cDNA and DNA sequence analysis. An oligo (dT)-primed human placental λgt11 cDNA library, kindly provided by Y. Ebina (22) was screened with polyclonal rabbit anti-human Factor XIII a-chain antibody (Hoechst Calbiochem, La Jolla, CA) using an immunoscreening kit, CLIK (Clontech, Palo Alto, CA) (23). Insert DNA from Eco RI digestion of the positive clones was ligated to an Eco RI-digested pSP64 vector (24). Plasmid DNA was prepared from transformed *Escherichia coli* HB101 cells and was purified by an alkaline-SDS method (25, 26). Restriction fragments from one of the positive clones, G25, were subcloned into phage M13mp18 and M13mp19. Single-stranded phage DNA templates were prepared as previously described (27). DNA sequences were determined by the dideoxynucleotide chain termination technique (28) using deoxyadenosine 5'-α-[^{35}S]thiotriphosphate (650 Ci/mmol; Amersham Corp.,) and the 17-mer universal primer.

Northern blot analysis. RNA was isolated from frozen placental tissue

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by homogenization in 5 M guanidine monothiocyanate followed by precipitation in 4 M LiCl (29). The poly $(A)^+$ mRNA fraction was selected by elution from an oligo (dT)-cellulose column (30). Poly $(A)^+$ mRNA was electrophoresed on a 1% agarose 2.2 M formaldehyde gel, blotted to nitrocellulose, and hybridized under high stringency conditions with ^{32}P-labeled Eco RI restriction fragments of the G25 clone: 630 base pair (bp), 950 bp, or 1.1 kb.

Protein purification and amino acid sequencing. Factor XIII a-chain was isolated from outdated platelets by published methods (31). The a-chains were cleaved with the protease V8, reduced and alkylated, and separated by high performance liquid chromatography (HPLC) on a Bio-Rad RP304 25 cm column. The fragments were subjected to sequence analysis using gas phase sequencer (Applied Biosystems, Foster City, CA).

Chromosome localization of the Factor XIII a-chain gene. The chromosomal location of the gene was analyzed by direct hybridization of the same 630 bp, 950 bp, and 1.1 kb probes, used for Northern blots, to the DNA of chromosomes resolved by dual laser chromosome sorting (32).

Results

The human placental λgt11 cDNA library was screened at high cell density with the polyclonal antibody to human Factor XIII a-chain. Of 200,000 recombinant phages, 44 clones reacting with the antibody were isolated. 20 clones were selected for secondary screening at lower plaque density, and all 20 again reacted with the antibody. Phage DNA from 12 of the 20 isolates was prepared and digested with Eco RI. The majority of the immunoreactive clones exhibited three Eco RI restriction fragments, of 630, 950, and 1,140 bp in length, although a few clones showed variant band patterns. The restriction map for one of the clones, G25, is shown in Fig. 1 *A*.

In order to confirm the authenticity of this immunoreactive clone, portions of the G25 cDNA were sequenced, and the derived amino acid sequence was compared to that obtained by direct amino acid sequencing of V8 cleavage fragments from purified platelet Factor XIII a-chain. The solid black bar in Fig. 1 *A* indicates the location of a sequence 81 nucleotides long, shown in line 1 of Fig. 1 *B*, whose derived amino acid sequence (line 2) is homologous to the direct peptide sequence from platelet Factor XIII a-chains (line 3). The direct amino acid sequence confirmed that the cDNA clone is Factor XIII a-chain. 21 of the 27-residues in the derived and direct amino acid sequences were identical. At four of the six discrepant residues, the direct peptide sequence had ambiguities. The major signal in the HPLC analysis differed from the derived amino acid; however, the minor HPLC signal in all four cases was identical to the nucleotide-derived amino acid. At the remaining two discrepant residues, methionine was derived from the nucleotide sequence, while direct amino acid sequencing indicated histidine residues. The most likely explanation is that methionine sulfone, an oxidized form of methionine in the HPLC system used for sequencing, has the same retention time as histidine.

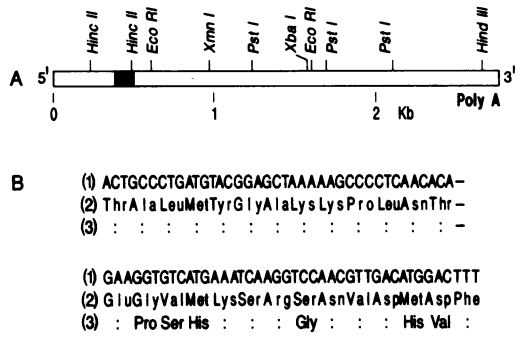

Figure 1. (*A*) Restriction endonuclease map of partial human placental Factor XIII a-chain cDNA. Solid black bar indicates location of sequence in B. (*B*) Nucleotide sequence (line 1) and its derived 27-residue amino acid sequence (line 2), homologous to the amino acid sequence (line 3) determined by direct peptide sequencing of purified platelet Factor XIII a-chains. : indicates identical amino acids found in both the derived and direct sequences.

By Northern blot analysis, each of the three Eco RI restriction fragments from clone G25 (630, 950, 1,140 bp), radiolabeled with ^{32}P, hybridized to a single human placental poly $(A)^+$ mRNA, estimated to be ~ 4.0 kb in length (ranging from 3.75 to 4.4 kb in different experiments). The 630-bp fragment, which includes the demonstrated homology to the Factor XIII a-chain amino acid sequence, hybridized (Fig. 2) to an identical size poly $(A)^+$ mRNA from U937 cells (human histiocytic lymphoma with monocytelike morphology, ATCC CRL 1593), both undifferentiated and differentiated with 1 mM dibutyryl cAMP for 48 h. Poly $(A)^+$ mRNA from HEL cells (human erythroleukemia with lymphoblastlike features, ATCC TIB 180) and K562 cells (human chronic myelogenous leukemia, ATCC CCL 243) showed no hybridization. HEL cells and K562 cells were chosen because they express platelet glycoproteins (33, 34), suggesting that both cell lines might synthesize Factor XIII a-chain, another platelet protein.

The chromosomal location of the Factor XIII a-chain gene was analyzed by direct hybridization of each of the three ^{32}P-radiolabeled G25 Eco RI restriction fragments to the DNA of chromosomes resolved by dual laser chromosome sorting (Fig. 3). The gene for Factor XIII a-chain mapped uniquely to chromosome 6 with each probe.

Discussion

Homology of the direct amino acid sequence of purified platelet Factor XIII a-chain with the nucleotide-derived amino acid sequence confirms that the immunoselected clone G25 is authentic cDNA for the Factor XIII a-chains. The Northern blot analysis of human placental poly $(A)^+$ mRNA shows that the message for Factor XIII a-chain is ~ 4.0 kb, using each of the three G25 Eco RI restriction fragments as probes. Since the cDNA clone

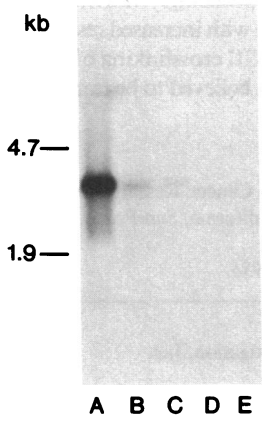

Figure 2. Identification of Factor XIII a-chain mRNA by Northern blot analysis. ^{32}P-Radiolabeled cDNA (630 bp restriction fragment) was hybridized to human poly $(A)^+$ mRNA from: (*lane A*) Human placenta (1.4 μg); (*lane B*) U937 cells, undifferentiated (8 μg); (*lane C*) U937 cells, differentiated with 1 mM dibutyryl cAMP for 48 h (4.4 μg); (*lane D*) HEL cells (4.4 μg); (*lane E*) K562 cells (5 μg).

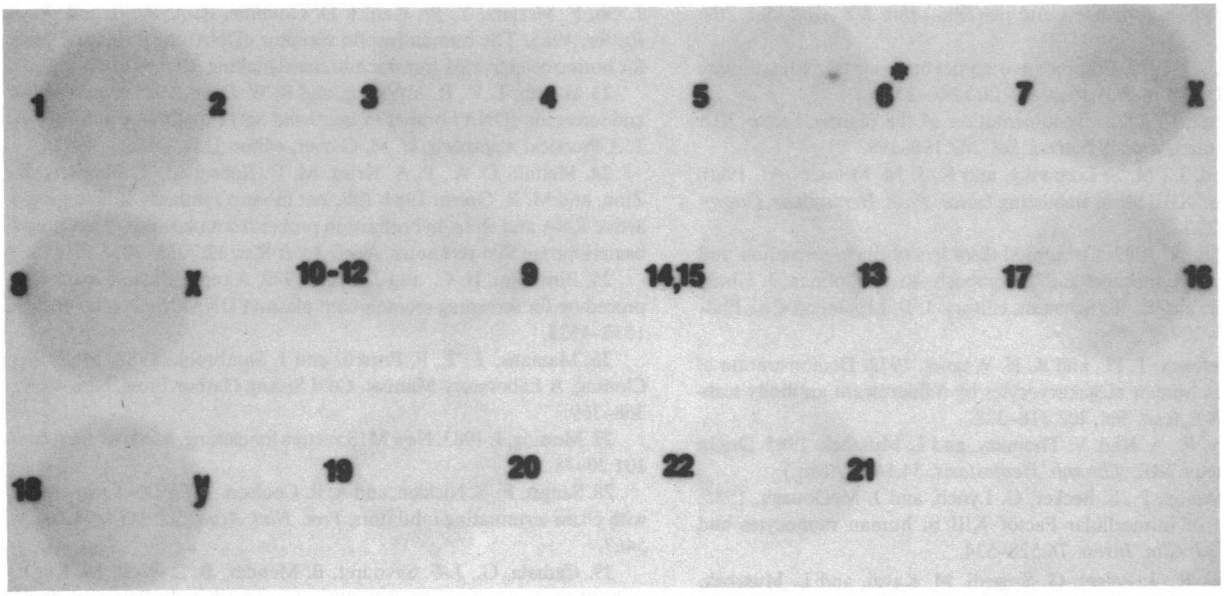

Figure 3. Autoradiogram of dot-blot filters of sorted chromosomes. ^{32}P-Radiolabeled cDNA (1.1 kb restriction fragment) was hybridized to the DNA of chromosomes resolved by dual laser chromosome sorting and bound to nitrocellulose filters. Identical results were obtained using ^{32}P-radiolabeled 630 bp and 950 bp probes.

G25 is 2.7 kb in length, this represents a portion of the complete cDNA. The region corresponding to the poly(A) tail of the mRNA is included in this cDNA (Fig. 1 *A*). Identifying the poly (A) tail by sequence analysis helped to orient the Eco RI restriction fragments in the λgt11 vector. The relative molecular weight (M_r) of Factor XIII a-chain is ~ 75,000, thus predicting a protein of ~ 660 amino acids, encoded by 1980 nucleotides. Since the message is ~ 4,000 nucleotides, a 1–2-kb untranslated region, most likely at the 3′ end, is predicted.

Factor XIII a-chain has been identified in human monocytes and macrophages by immunoblotting and immunohistochemical techniques (15, 16). Detecting protein in these cells, however, does not exclude the possibility that the a-chains were taken up from the plasma rather than synthesized within the cells.

The 630 bp cDNA restriction fragment hybridizes on Northern blot (Fig. 2) to differentiated and undifferentiated U937 cell mRNA of identical size as that identified for human placenta. Although less placental poly $(A)^+$ mRNA was applied to the gel compared to the other samples, hybridization was most intense in this lane, reflecting the greater relative abundance of message for Factor XIII a-chain in the placenta. These results demonstrate that in addition to its production by placenta, Factor XIII a-chain is synthesized in monocytelike cells. The function of Factor XIII in these cells has not yet been determined.

Each of the three G25 Eco RI restriction fragments, radiolabeled with ^{32}P, hybridized uniquely to chromosome 6 on the chromosomal dot blot (Fig. 3). These results agree with those of Board (19) and Olaisen (20), who reported linkage of Factor XIII a-chain to the HLA-region genes on chromosome 6, and provide more definitive verification of the chromosomal localization.

In addition to being essential for normal hemostasis, Factor XIII a-chain is essential for a wide variety of tissue and organ functions. The availability of Factor XIII a-chain cDNA will permit investigations into the regulation of synthesis of this important clotting factor.

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