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Factor XI protein in human pancreas and kidney

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Dear Sir,

Factor XI (FXI) is the zymogen of a plasma protease that contributes to haemostasis through activation of factor IX (1). As with other coagulation proteases, the liver is the primary source of plasma FXI (2–4). However, we have detected FXI mRNA by northern blot in human pancreas and kidney (4). The signal for pancreas was of comparable intensity to that of liver. Subsequently, we isolated cDNAs from a human pancreas library (not shown) with identical nucleotide sequence to the FXI cDNA from liver (5). We now present results of a study demonstrating FXI protein in human pancreas and kidney.

Paraffin-embedded sections of human liver, pancreas and kidney (three specimens for each) were obtained from surgery and autopsy specimens used as normal controls in the Vanderbilt University Hospital Histology Laboratory. Specimens were heated to 60°C for 1 hour (h), fixed sequentially with xylene, 100% ethanol, 3% H₂O₂ in 100% methanol, and 95% and 75% ethanol, then rinsed with water. Antigen retrieval was with 10 mM citrate pH 6.0 for 1 minute (min), and steaming for 25 min. Slides were incubated with blocking buffer (1.5% horse serum in phosphate-buffered saline [PBS]) for 20 min, then mouse anti-human FXI monoclonal IgG 1A6 (5 µg/ml) in blocking buffer at RT for 1 h. 1A6 binds to the FXI A2 domain, and recognizes a single 160 kDa protein in plasma corresponding to FXI (6). After washing three times with PBS, sections were incubated with biotinylated anti-mouse IgG (Vectastain Elite ABC, Vector Laboratories, Burlingame, CA, USA) in blocking buffer, and developed according to the manufacturer's recommendation. Sections were counterstained with hematoxylin, and treated with 1.2% lithium carbonate to enhance blue color.

Representative photomicrographs are shown in Figure 1. In liver, as expected, the cytoplasm of hepatocytes, but not cells in hepatic sinusoids, stained uniformly for FXI (Fig. 1B). Intense staining was detected in pancreatic islets (Fig. 1D and E), but not in acinar cells of the exocrine pancreas. This is surprising, considering the strong signal intensity for FXI in northern blots (4), and the fact that islets make up only 1–2% of pancreatic tissue. FXI was also present in tumor cells from two islet cell carcinomas (insulinomas – Fig. 1G and H). In kidney, FXI was detected in cells of the proximal and distal tubules (Fig. 1J), and the collecting ducts (not seen in Fig. 1J), but not in glomeruli. Identical staining was observed for all tissues with goat

polyclonal antihuman FXI IgG (Affinity Biologicals, Ancaster, Ontario, Canada – data not shown). FXI protein was not detected in muscle, small bowel, lymph node, or bone marrow (data not shown).

This study demonstrates for the first time that FXI protein is synthesized outside of the liver. Patients with congenital FXI deficiency may bleed excessively with trauma, particularly when the urinary tract or oropharynx is involved (1). It is thought that FXI contributes to maintenance of clot integrity in the face of robust fibrinolysis in these tissues. FXI synthesized in the kidney may help maintain local haemostatic balance by countering fibrinolytic activity associated with high local levels of urokinase. A role for FXI in islet cell physiology has not been identified and, at this point, is not obvious. Infusion of pancreatic islets into the portal vein during islet cell transplantation for diabetes mellitus is accompanied by a thrombo-inflammatory response (7) that occasionally causes disseminated intravascular coagulation (8) or portal vein thrombosis (9). High plasma levels of factor XI are a risk factor for venous thromboembolism (10), and it is possible that FXI may contribute to the pro-coagulant state during islet cell transplants.

The transcription factor hepatocyte nuclear factor-4 α (HNF-4 α) is required for liver-specific FXI expression (2). Hepatocytes and pancreatic islet cells share similarities in terms of developmental paradigms and transcriptional control of gene regulation (11). HNF-4 α is required for normal glucose-stimulated secretion of insulin by β -cells (12). HNF-4 α is also expressed in kidney (13), reinforcing the impression that the tissue distribution of FXI protein synthesis is related to expression of this transcription factor. FXI is structurally similar to plasma prekallikrein (PK) (5). The genes for the two proteins are separated by <10 kilobases, and are clearly products of a duplication event involving an ancestral gene (2). PK mRNA is present in many human tissues, with the highest levels in liver, pancreas and kidney. PK protein was recently demonstrated in hepatocytes, pancreatic islets, and kidney tubules and collecting ducts (14); a distribution strikingly similar to that of FXI. It is not known if HNF-4 α is required for PK gene expression, but the results suggest that FXI and PK expression may be coordinated in some tissues.

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Figure 1. Detection of FXI protein in normal human tissues and islet cell tumors

Immunostaining was performed on mounted thin sections of human tissues, using the anti-human FXI monoclonal IgG 1A6, as described under Materials and methods. A, C, F and I) Controls in which no primary antibody (1A6) was used. A and B) Normal liver (40X magnification); C–E) Normal pancreas (C and E 40X, D 20X); F–H) Islet cell tumors (20X); I and J) Normal kidney (40X). E) Magnification of the field in panel D outlined by the box. Images were made with an Olympus BX41 microscope fitted with a DP70 camera using Olympus DP controller software.