Supplemental data

Production of monoclonal anti-FXIII-B antibodies

8-12 weeks old Balb/c mice were immunized subcutaneously with 100 µg plasma FXIII-B emulsified in complete Freud’s adjuvant. After two weeks 50 µg antigen adsorbed on aluminium hydroxide gel particles was injected intraperitoneally as booster, which was repeated following another two-week interval. Four days before fusion a final boost of 50 µg antigen was introduced intraperitoneally. Immune spleen cells were fused with Sp-2/o myeloma cells according to a standard protocol. The culture supernatants were first tested for reaction with FXIII-B<sub>2</sub> coated to the surface of a microplate. Antibody binding was detected by horse radish peroxidase (HRPO) labeled anti-mouse IgG.

Positive supernatants were selected for the inhibition of complex formation between FXIII-A<sub>2</sub> and FXIII-B<sub>2</sub> in an ELISA-type assay that included the following steps:

1) Microtiter plates were coated with a monoclonal anti-FXIII-B antibody that reacted with FXIII-A<sub>2</sub>B<sub>2</sub> and free FXIII-B<sub>2</sub> equally well. 50 µL antibody (1 µg/mL in 0.1 M carbonate buffer, pH 9.6) was incubated in the microplate overnight at +4°C.

2) The plates were blocked by incubation with 100 µL 0.5% bovine serum albumin (BSA) in phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST-BSA) and 0.5 M NaCl.

3) Then, the antibody immobilized to the plate surface was incubated with 50 µL FXIII-B<sub>2</sub> (100 ng/mL in PBST-BSA).
4) In the next step 50 µL hybridoma supernatants or buffer was added to the wells containing FXIII-B₂ fixed to the plate surface through the antibody.

5) Finally, the wells were incubated with 50 µL FXIII-A₂ (100 ng/mL in PBST-BSA) and the formation of FXIII-A₂B₂ complex was detected by HRPO-labeled monoclonal anti-FXIII-A antibody.

With the exception of coating, each incubation step was carried out at 23 °C for 60 min and it was followed by extensive washing with PBST. The amount of bound HRPO-conjugated antibody, which was the measure of complex formation, was determined by tetramethyl-benzidene substrate at 450 nm.

Hybridomas producing antibodies that inhibited or not inhibited complex formation were selected and cloned by limiting dilution technique. Two antibodies, anti-FXIII-B(1) that did not inhibit complex formation and anti-FXIII-B(2) that inhibited complex formation were isolated from culture supernatants by Protein G affinity chromatography and used in the experiments.

References