Brief report

Factor XIII A subunit–deficient mice developed severe uterine bleeding events and subsequent spontaneous miscarriages

Shiori Koseki-Kuno, Mitsunori Yamakawa, Gerhard Dickneite, and Akitada Ichinose

To understand the molecular pathology of factor XIII (FXIII) deficiency in vivo, its A subunit (FXIIIA)–knockout (KO) mice were functionally analyzed. Although homozygous FXIIIA female KO mice were capable of becoming pregnant, most of them died due to excessive vaginal bleeding during gestation. Abdominal incisions revealed that the uteri of the dead mice were filled with blood and that some embryos were much smaller than others within a single uterus. A series of histologic examinations of the pregnant animals suggested that massive placental hemorrhage and subsequent necrosis developed in the uteri of the FXIIIA KO mice on day 10 of gestation. This was true regardless of the genotypes of fetuses. These results are reminiscent of spontaneous miscarriage in pregnant humans with FXIII deficiency and indicate that maternal FXIII plays a critical role in uterine hemostasis and maintenance of the placenta during gestation. (Blood. 2003;102:4410-4412)

Introduction

Coagulation factor XIII (FXIII) is a heterotetramer consisting of A subunits (FXIIIA) and B subunits (FXIIIB). FXIII catalyzes intermolecular cross-linking reactions between fibrin monomers, α₂-plasmin inhibitor, and fibronectin. These reactions increase the mechanical strength of the fibrin clot and its resistance to proteolytic degradation and enhance the assembly of the extracellular matrix. Accordingly, a deficiency of FXIII results in defective cross-linking reactions of these substrates and is thus associated with a number of diseases. Congenital FXIII deficiency is caused by defects in the F13A or F13B genes, leading to a bleeding tendency and abnormal wound healing in affected patients and spontaneous miscarriage in female patients. To understand the molecular pathology of these deficiencies, we have identified a number of mutations in the F13A and F13B genes in patients’ DNA and have analyzed the molecular mechanisms of FXIII deficiency using in vitro procedures. Because it is not possible to understand completely the clinical pathologic mechanisms of this disease in vivo, we performed functional analyses on FXIIIA knockout (KO) mice.

Study design

FXIIIA KO mice were created by replacing exon VII with a neomycin-resistance cassette as described previously. All KO and wild-type CBA mice were housed in a specific pathogen-free facility. Experimental procedures were approved by the Animal Care and Use Committee of Yamagata University and were carried out in accordance with the guidelines of this committee and that of Japanese governmental law.

Using tail biopsy DNA, genotyping of the mice was performed by polymerase chain reaction (PCR) with 2 forward primers designed from the neomycin-resistant gene (5’-CAC TGC ATT CTA GTT GTG GTT TGT CC-3’) and exon VII of the mouse F13A gene (5’-GCC AAG GAT GAT GAA GGT GTT CTT-3’), respectively, and a common reverse primer from intron G (5’-CCC TGA GAC TTA CGG AAG-3’). Tissues collected for histologic analyses were placed in 10% neutral-buffered formalin, processed into paraffin, and 3-μm sections were prepared and stained with hematoxylin and eosin by standard techniques.

The distribution of events between the study groups was analyzed using 4 × 2 contingency tables for individual study groups as well as 2 × 2 tables for a combined group of female wild-type mice and female homozygous FXIIIA KO mice. The χ² test was also carried out by using StatView software (Abacus Concepts, Berkeley, CA). Differences were considered statistically significant at P values less than .05.

Results and discussion

Western blot analysis and amine-incorporation enzymatic assay confirmed the complete absence of FXIIIA in the uterus and plasma (where FXIIIA is normally expressed/present) of the homozygous FXIIIA KO mice identified by genotyping (data not shown). Despite the complete loss of FXIII, all homozygous FXIIIA KO mice had appeared normal at birth as previously reported. Whereas all 20 of the female homozygous KO mice survived, 3 of 20 male KO mice died of massive intrapleural bleeding within 6
months after birth, which is likely due to the male tendency to fight with one another.

However, a female homozygous KO mouse died of excessive spontaneous bleeding from its vagina 2 weeks after mating with a wild-type male CBA mouse. This symptom had not been noted when heterozygous KO mice were sib-mated in a laboratory of the German investigator (G.D.).

Accordingly, 4 types of mating pairs were tested to explore fertile capability (Table 1). Notably, female KO mice developed overt genital bleeding spontaneously and frequently around the tenth day of gestation (10 dG), whereas none of the wild-type female mice bled. About half of the pregnant female KO mice died of massive genital bleeding, although all wild-type female mice survived. This was true regardless of the genotype of male mating partners.

There was no marked difference in the number of pregnancies between the KO and wild-type mice, whereas parturition was much less in the KO mice than in the wild-type mice. In addition, bleeding events and miscarriage occurred more often in the KO mice. These results suggested that, although conception would be normal, maintenance of pregnancy would be defective in the FXIIIa KO mice.

Abdominal incisions uniformly revealed that the uteri and vaginas of the dead female mice were filled with blood and the sizes of embryos/fetuses were uneven (Figure 1A-B). Microscopic examination of these mice uniformly revealed evidence of varying sizes of bleeding and hemorrhagic necrosis in the placentas for small embryos, whereas standard-sized embryos showed only signs of microhemorrhage (data not shown).

To confirm that intrauterine bleeding develops prior to maternal death, histologic analyses of 10 homozygous KO and 4 wild-type mice were performed after they were killed on 10 dG regardless of the presence or absence of overt vaginal bleeding. Blood pools of variable sizes were uniformly observed in the uterine cavity for a number of embryos in all killed pregnant KO mice. One pregnant KO mouse showed severe bleeding in the uterine cavity for many embryos, whereas another showed only mild bleeding for a single embryo (Figure 1C-D). In the latter, the blood pool in the uterine cavity was in direct contact with the placenta (Figure 1E red circle). In magnified views, the blood pool was just in contact with the labyrinthine zone (Figure 1F area I) and an area of hemorrhagic necrosis (Figure 1F area II).

It is very likely that a chance vascular breakage in the placenta leads to hemorrhage because the occurrence and severity of the bleeding events and the number of abortions were quite variable between individual KO mice. In addition, the number of pups varied from 1 to 9 among female KO mice, indicating that severe hemorrhage in the placenta resulted in intrauterine fetal death, whereas mild bleeding led to survival of embryos even in the same uterus of a single mouse. The presence of FXIIIa in the circulating blood of the labyrinthine layer seems to be critical for maintenance of the placenta by preventing excessive bleeding.

Overt vaginal bleeding uniformly developed at around 10 dG, the time when embryonic trophoblasts are invading and disrupting maternal vasculature within the labyrinthine layer of the placenta.

Table 1. Ratios of death and abortion in relation to genital bleeding events

<table>
<thead>
<tr>
<th>Mating pairs</th>
<th>Gender/genotype</th>
<th>No. female</th>
<th>No. bled (%)</th>
<th>No. dead (%)</th>
<th>Total no. pregnancy (average)</th>
<th>No. overt bleeding (%)</th>
<th>No. abortion (%)</th>
<th>No. parturition (average)</th>
<th>No. pups (average)</th>
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<tr>
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<td>8</td>
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<td>0 (0)</td>
<td>46 (5.8)</td>
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<td>2 (4)</td>
<td>44 (5.5)</td>
<td>279 (6.3)</td>
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<tr>
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<td>10</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>63 (6.3)</td>
<td>0 (0)</td>
<td>2 (3)</td>
<td>61 (6.1)</td>
<td>348 (5.7)</td>
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<td>4 (44)</td>
<td>4 (44)</td>
<td>33 (3.7)</td>
<td>8 (24)</td>
<td>12 (36)</td>
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<td>93 (5.5)</td>
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<tr>
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<td>10</td>
<td>10 (100)</td>
<td>7 (70)</td>
<td>41 (4.1)</td>
<td>26 (63)</td>
<td>15 (37)</td>
<td>19 (1.9)</td>
<td>44 (2.3)</td>
</tr>
</tbody>
</table>

*Four animals died during pregnancy.
†Seven animals died during pregnancy.

Figure 1. Intrauterine bleeding occurred in female homozygous FXIIIa KO mice. (A-B) Overview of the uteri of FXIIIa KO mice that spontaneously died of excessive vaginal bleeding. Sizes of embryos were uneven within the same uterus of a single animal (A). Both uterine horns were filled with blood and degenerating placentas (B). (C-D) Bleeding in the uterine cavity of KO mice killed at 10 dG. Both uterine horns for most embryos showed severe bleeding in one mouse (C), whereas a uterine shell for a single embryo in another mouse had a pool of blood (D). A bold line shows the border of excision for histologic examination. (E) The portion surrounded by a red circle is magnified in panel F (original magnification, × 40 [panel E] and × 200 [panel F]). (F) There is a region of hemorrhagic necrosis (area II) in the labyrinth adjacent to a blood pool (area I).
The fact that intrauterine bleeding/miscarriage/death occurred only in homozygous FXIII female KO mice strongly suggests that hemorrhage in these KO mice was from a maternal source. This is consistent with the observation that blood pools were found only in the uterine cavity and not in the amniotic cavity and fetus itself. In addition, homozygous FXIIIA KO embryos/fetuses have been shown to develop normally and have been produced even by homozygous KO mothers, as long as their placentas and uteri are intact. Therefore, a complete lack of FXIIIA in embryos/fetuses does not contribute significantly to the intrauterine bleeding/miscarriage of FXIIIA KO mice. This concept is also supported by the fact that substitution therapy with FXIII concentrates permitted normal pregnancies in human patients with FXIII deficiency.\(^\text{18-20}\)

Homozygous fibrinogen-deficient mice also displayed miscarriage\(^\text{21,22}\) because of intrauterine bleeding around 10 dG. Again, it is suggested that maternal fibrinogen plays an essential role in the maintenance of pregnancy.\(^\text{22}\)

**Acknowledgments**

We are grateful to Dr P. Bishop for providing an affinity-purified antibody against FXIII; Drs M. Souri, K. Ohwada, and E. Kamimura, and Mr T. Ito of Yamagata University and Drs T. Kobayashi and T. Iwaki of Hamamatsu Medical College for helpful discussion; and Ms L. Boba for her help in preparation of the manuscript.

**References**

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