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Successful Umbilical Cord Blood Transplantation for Fanconi Anemia Using Preimplantation Genetic Diagnosis for HLA-Matched Donor

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Fanconi anemia is a rare autosomal recessive disease characterized by bone marrow failure, developmental anomalies, and a high incidence of myelodysplasia and acute myeloid leukemia. Stem cell transplantation is the only curative treatment. In the absence of matched- sibling donor, an alternative mismatched family or matched unrelated donor can be used, but the results are inferior to the matched-sibling transplant and carry a high risk of morbidity and mortality. Preimplantation genetic diagnosis (PGD) has been increasingly used in recent years for mutation analysis for many genetic disorders and results in the birth of healthy children, saving the need for the termination of pregnancy of an affected embryo. The use of PGD for combined analysis of mutation and HLA-matching was reported for the first time in 2001. This enables the birth of an unaffected child who can serve as a donor for an affected sibling in need for stem cell transplantation. We report successful cord blood transplantation for a Fanconi anemia patient from his HLA-matched sibling, born after PGD that included mutation analysis for Fanconi anemia and HLA typing. PGD can provide an unaffected donor for a sibling affected by genetic disease in the absence of a compatible related donor. Am. J. Hematol. 77:397–399, 2004. © 2004 Wiley-Liss, Inc.

Key words: preimplantation genetic diagnosis; Fanconi anemia; stem cell transplantation

INTRODUCTION

Preimplantation genetic diagnosis (PGD) is an evolving technique that provides a practical alternative to prenatal diagnosis. The advantage of PGD is that the genetic testing is performed on embryos before a clinical pregnancy is established, thus avoiding termination of pregnancy [1]. This technique is applied for couples that are at substantial risk of transmitting a serious genetic disorder to their offspring or for detection of chromosomal abnormalities. Samples for genetic testing are obtained from blastomeres after in vitro fertilization, and only embryos that are not affected by the genetic disorder are transferred to the uterus.

The first live birth following PGD for cystic fibrosis was reported in 1992 [2]. Since then, PGD has been performed for a variety of genetic disorders, including Tay-Sachs disease, sickle cell anemia, thalassemia, and

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phenylketonuria as well as for diagnosis for cancer predisposition (p53 tumor suppressor gene) [1,3–7]. Diagnostic procedures include techniques that detect abnormalities in the number or structure of the chromosomes, like fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) [8]. Another technique is based on amplification of the gene of interest by polymerase chain reaction (PCR) followed by sequencing that determines the specific mutation. Diagnostic tests are performed on single cells or double cells. This necessitates highly qualified laboratory skills especially with regard to single-cell PCR.

Fanconi anemia is an autosomal recessive disorder characterized by diverse congenital abnormalities, progressive bone marrow failure, and increased risk of developing myelodysplasia, acute myeloid leukemia, and various epithelial malignancies [9,10].

Stem cell transplantation is the only curative treatment for restoring normal hematopoiesis and preventing the occurrence of leukemia [11].

Umbilical cord blood is an attractive alternative source of hematopoietic stem cells [12]. Following the pioneered cord blood transplantation (CBT) by Gluckman et al. [13] in a child with Fanconi anemia, multiple successful CBT were performed for this indication, including unrelated cord blood. The use of fludarabine as part of the conditioning regimen improved the results in the unrelated setting [14].

Parents who already have a child affected with Fanconi anemia face a serious dilemma: they would like to make sure that their next children will be free of disease while at the same time serve as a matched donor for their affected child.

Combined preimplantation genetic diagnosis and HLA typing seems as an attractive option to achieve this goal.

The first PGD for Fanconi anemia combined with HLA-matching was reported by Verlinsky et al. [15]. The transplantation course of this child was recently described [16].

We report the second successful PGD for Fanconi anemia child combined with HLA typing.

Umbilical cord blood was transplanted to the affected sibling resulting in a successful hematopoietic reconstitution.

CASE REPORT

A 3.5-year-old boy with Fanconi anemia was referred to our department for umbilical cord blood transplantation from his HLA-matched sister.

He was the first child to his unrelated parents from Ashkenazi descent. Intrauterine growth retardation had been noted at 28 weeks of gestation, and he was born at 36 weeks with low birth weight. His medical history was significant for cardiac abnormalities including VSD and PDA, which was partially repaired at the age of 1 year. Other congenital abnormalities suggestive of Fanconi anemia were bilateral hypoplastic thumbs, bilateral radial dysplasia, and micro-ophthalmia. A diagnosis of Fanconi anemia was established at the age of 6 months based on increased chromosomal breakage induced by diepoxybutane (DEB). Molecular testing showed that he was homozygous for the common Ashkenazi Jewish mutation in the *FANCC* gene: IVS 4 + 4 A→T.

During the year prior to the transplant the patient's hematologic parameters revealed persistent thrombocytopenia ([16–25] $\times$ 10^9/L), hemoglobin levels of 8.5–10.9 g% and ANC of (0.4–1.9) $\times$ 10^9/L. The bone marrow was hypocellular with dyserythropoietic changes but without clonal abnormalities. He was transfusion independent and was not treated with any growth factors or androgens.

Since he had no matched sibling donor, a search for unrelated donor located a full matched donor for him. However, since the results for stem cell transplantation for Fanconi anemia patients from unrelated donors were inferior to matched sibling donor, the parents decided to try another approach. They attempted PGD for Fanconi anemia combined with HLA-typing of the embryo in order to have an unaffected child who could serve as a potential donor.

After 3 cycles, the transfer in the last cycle resulted in a clinical pregnancy and the birth of a healthy carrier of the IVS4 mutation in the *FANCC* gene. The results of both mutation analysis and HLA-matching were confirmed by chorionic villous sampling. Umbilical cord blood was collected at the time of birth and was frozen for future use.

When the donor was 4 months old, a preparation for transplantation was commenced. The patient was conditioned with fludarabine 25 mg/m^2/day for 5 doses (days -7 to -3) and cyclophosphamide 10 mg/kg/day for 4 doses (days -5 to -2). GVHD prophylaxis was cyclosporine only.

At the day of transplant he received 7.6 $\times$ 10^7 nucleated cells/kg and 2 $\times$ 10^5 CD34/kg.

The course was uneventful except for *Staphylococcus aureus* bacteremia, which necessitated removal of the central line catheter. Engraftment of neutrophils occurred at day 15 post-transplant, and time for platelet transfusion independence was 37 days. He was discharged at day 21 and was followed in the outpatient clinic. There was no evidence of acute or chronic GVHD. The boy is now 1.5 years post-transplant in very good general condition with normal blood

counts and evidence of full-donor chimerism in repeated FISH studies.

DISCUSSION

We report a continuous process that started with PGD for Fanconi anemia combined with HLA matching and followed by transplantation of cord blood stem cells collected at birth from the healthy HLA-matched newborn sibling of the affected brother.

There are unique characteristics of Fanconi anemia that deserve specific consideration and may emphasize the importance of PGD in this disease. As already stated, stem cell transplantation is the only curative treatment for Fanconi anemia, and the preferable donor is an HLA-matched sibling. In the absence of matched sibling, using an alternative donor such as mismatched family donor or matched unrelated donor carries a substantial risk of transplant-related complications [17]. Fanconi anemia patients are at particular at risk due to severe DNA repair defects, which make them more susceptible to the conditioning regimen.

Although most people will agree that in this particular case there was a full justification for embryo selection, PGD in general poses some serious ethical problems.

Discarding of healthy HLA non-identical embryo may be a threat to those believing that the penetration of the oocyte by the sperm symbolizes the beginning of life. This concern may be also raised in the usual process of IVF as it involves the discard of embryos. Only 2–3 embryos are transferred to the uterus whereas the rest are cryopreserved but never used again.

Another concern is that developing techniques that enable the creating of a child with specific genetic traits currently used as preventive medicine may be used for designing genetics.

In view of the above-mentioned reservation, we believe that PGD for the detection of genetic and chromosomal abnormalities as well as for the benefit of an affected relative should be performed under strict supervision of national and ethics committees.

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