RAPID COMMUNICATION

Clonal, Nonconstitutional Rearrangements of the MLL Gene in Infant Twins With Acute Lymphoblastic Leukemia: In Utero Chromosome Rearrangement of 11q23

Rearrangements of chromosome band 11q23 are common in infant leukemias, comprising more than 70% of the observed chromosome abnormalities in children less than 1 year of age. The MLL gene, which is located at the 11q23 breakpoint in infant, childhood, and adult acute leukemias, has been cloned and has homology to the Drosophila trithorax gene. The breakpoints in MLL are restricted to an 8.3-kilobase pair (kb) region of the gene that is involved in translocations with as many as 29 other chromosomal regions in a number of phenotypically distinct acute leukemias. We have detected an identical, clonal, nonconstitutional rearrangement of the MLL gene in peripheral blood cells from a pair of female infant twins with acute lymphoblastic leukemia (ALL) and a t(11;19)(q23;p13.3). The detection of nonidentical IGH rearrangements suggests that the MLL rearrangement took place in a B-cell precursor or hematopoietic stem cell in one twin which was transferred in utero to the other fetus resulting in ALL with an identical aneuploid karyotype in both infants. We speculate that other MLL-related infant leukemias may also develop in utero, and that the rearrangements may occur consistently in stem cells or early precursor cells, accounting for the frequency of mixed-lineage leukemia in infants.

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would be only a 50% probability that they shared identical alleles for each heterozygous parental RFLP. In addition to the eight common markers, the twins had the identical inheritance of the father's X chromosome and the same ABO blood group. When all of these results were taken into account, the posterior probability of monozygosity was estimated to be 0.998. In the event that the remission blood specimen from twin B was the result of the colonization of her hematopoietic system with cells of twin A, the calculation of zygosity could be in error.

Initial cytogenetic analysis of leukemia samples from the infants showed that both had terminal deletions at 11q23. Subsequent analysis of a leukemia sample from twin B by fluorescence in situ hybridization (FISH) with probes which were distal to the \textit{MLL} locus on 11q23 showed that the chromosome abnormality was a t(11;19)(q23;p13.3). Other translocations have been identified in leukemia samples initially thought to have 11q23 deletions with this strategy.\textsuperscript{20} DNA was extracted from leukemic blood from both twins, digested with \textsc{BamH}I or \textsc{EcoR}V restriction endonuclease, and separated by agarose gel electrophoresis. The DNA was transferred to a GeneScreen Plus nylon membrane (New England Nuclear, Boston, MA) and hybridized to the \textit{MLL.07B} cDNA probe. Two identical rearranged bands were detected in the leukemia samples, whereas only the 8.3-kb germline band was present in the remission sample B. Southern blot of \textit{Hind}III-digested DNA from peripheral blood taken from Twin A (a) and Twin B (b) at diagnosis of ALL and from normal placental DNA (C). The blot was hybridized to an Ig \textit{J} \textit{H} gene probe. Twin B shows two predominant rearranged bands; twin A shows \( \geq 6 \) rearranged bands. The common \textit{Hind}III band that migrates slightly above the rearranged bands in twin B was also observed as a faint band in the control, but may not be visible in this reproduction. It is probably due to nonspecific cross-hybridization.

The observation of the identical rearrangement of the \textit{MLL} gene in leukemia cells of both infants and the absence of the rearrangement in twin B's remission sample indicates that the rearrangement took place in a cell in one of the twins and that the clone was transferred in utero to the other. Transfer from twin A to twin B is likely because ALL developed earlier in twin A than in twin B. Southern blot analysis with a \textit{J} \textit{H} probe, performed on samples obtained at the time of diagnosis of ALL, showed nonidentical \textit{Ig} heavy chain gene (\textit{IGH}) rearrangements.\textsuperscript{21} Twin A showed \( \geq 6 \) nongermline bands in \textit{Hind}III-digested DNA, whereas twin B had two predominant rearranged bands with this restriction enzyme (Fig 1B). This suggests that the translocation event occurred in a pre-pre-B cell stage or in an earlier stem cell, and that independent rearrangement of the \textit{IGH} genes occurred after the translocation involving \textit{MLL} giving rise to unique clones in the two infants. Although less likely, it is possible that the \textit{MLL} rearrangement took place in a cell that had one or two \textit{IGH} rearrangements and that later underwent further rearrangement, perhaps via somatic hypermutation or \( V_{i1}-V_{i2} \) substitution. A study of infant B-cell ALL\textsuperscript{22} argues against such an explanation. In either case, multiple \textit{IGH} rearrangements are a common feature of infant leukemia and are associated with a poor prognosis.\textsuperscript{23}

Cases of leukemia in twins have been described for almost 30 years. The high concordance rate of leukemia (20% to 25%) specifically in monozygotic twins raised the suspicion of a transformation event in utero even in the earliest studies.\textsuperscript{24,25} Others have simultaneously reported concordant leukemia in three pairs of monozygotic infant twins, each pair with a clonal, nonconstitutional rearrangement of the \textit{MLL} (\textit{HRX}) gene.\textsuperscript{26} These two most recent studies suggest
that the chromosome rearrangements involving MLL, that are so frequently observed in infant acute leukemia in single births probably also occur in utero. Depending on the length of the latent period, the leukemia is observed as a congenital disease or develops within the first year of life. Rearrangements of 11q23 are observed in adult acute leukemias as well as in infant and childhood disease. In addition, these abnormalities occur in t-AML and t-ALL after treatment with topoisomerase II inhibitors. It is unclear whether the illegitimate recombination event involving MLL that occurs in utero is the same as that which occurs in adults.

Ford et al. have reported that the three pairs of twins in their study all had single, monochorionic placentas and that the transfer of malignant cells between fetuses can occur only if the placental circulation is shared. The infant twins described here, although also monochorionic, had separate, dichorionic placentas. Therefore, we have shown that the transfer of leukemia cells can occur in dichorial/dichorionic twins as well. In our case the transfer of the malignant clone could have occurred by crossing to the maternal circulation and back to the second twin. Small numbers of fetal cells are detectable in the maternal circulation in most women during pregnancy or after delivery. Both fetal lymphocytes and nucleated erythrocytes have been observed in maternal blood. Fetal red blood cells of compatible ABO groups have a life span similar to that of adult cells and in certain cases fetal cells have been detected in the mother years after the birth of the infant. Even ABO-incompatible fetal cells can survive weeks in the maternal circulation. More likely, the transfer may have occurred as the result of placental anastomosis. Anastomoses are common between monochorionic placenta in twin pregnancies but are extremely rare in dichorionic twins. Approximately 20 cases have been reported in which dizygotic twins have apparently exchanged blood cells in utero. In these rare exchanges blood precursor cells of each twin colonize the bone marrow of the other, resulting in hematopoietic chimeras. It is assumed that the duration of the anastomosis determines the percentage of "foreign" cells found in each twin and that immune tolerance allows the survival of the cells. To our knowledge, no other cases of monoclonal leukemia in dichorionic twins have been reported to date. However, the phenomena of blood chimeras in twins supports our hypothesis of transfer of an early B-cell precursor with an MLL rearrangement from twin A to twin B.

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