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Prenatal Origin of Chromosomal Translocations in Acute Childhood Leukemia: Implications and Future Directions

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We, and others, have demonstrated an in utero origin for translocations associated with childhood leukemia, with latency periods in some cases exceeding 10 years. The mechanism of generation of most of the translocations is thought to be aberrant repair following abortive apoptosis, rather than V(D)J recombination or exposure to topoisomerase II inhibitors. Folate supplementation may prevent some of the chromosome breakage leading to translocation formation. Translocations t(8;21) and t(12;21) have been shown to occur in the normal population (before birth) at a frequency that is 100-fold greater than the risk of developing the corresponding leukemia. In most instances, additional genetic changes are required for progression to leukemia. Tyrosine kinase receptor (RTK) mutations, which give cells a survival/proliferative advantage, are proposed to act cooperatively with fusion genes, leading to transformation. However, translocations and cooperating RTK mutations have not been identified for all leukemia subtypes, particularly in acute myeloid leukemia. The core binding transcriptional pathway is frequently targeted by translocation in utero. We propose that this pathway is highly sensitive during fetal hematopoiesis and may be targeted by mechanisms other than translocation. For each leukemia subtype it is important to characterize the corresponding leukemic stem cell, which is thought to be the initial target for translocation. This would help to elucidate the molecular pathways involved in the progression from preleukemic clone harboring a translocation to fully disseminated leukemia. Am. J. Hematol. 75:254–257, 2004. © 2004 Wiley-Liss, Inc.

Key words: childhood leukemia; translocation; in utero; stem cell

PRENATAL ORIGIN OF LEUKEMIC TRANSLOCATIONS

We recently reported that the fusion genes *PML–RARA* and *CBFB–MYHII*, characteristic of pediatric acute myeloid leukemia (AML) with t(15;17) and inv (16) respectively, can originate *in utero* [1]. Although the numbers in our study were small, this is an important proof-of-principle which brings the number of different pediatric acute leukemia-associated chromosomal rearrangements (including hyperdiploidy), with demonstrated prenatal origin, to six in different populations [2]. Three of the patients described in these studies presented with leukemia after age 10 [1,3]. While t(12;21), characteristic of common acute lymphoblastic leukemia (c-ALL), has a peak incidence in children 2–5 years of age [4], other translocations of demonstrated prenatal origin such as t(8;21), t(15;17), and inv(16), characteristic of different AML subtypes, occur more frequently in young adults (age 20–40) [5]. It remains to be determined if leukemias presenting in young adulthood with these translocations also have a prenatal origin.

MECHANISM OF TRANSLOCATION GENERATION

It is not currently known by what mechanism translocations are generated in utero. These translocations

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do not show the hallmarks of V(D)J recombination (a constitutive mechanism for rearrangement of somatic chromosomal DNA) [2]. Topoisomerase II is an enzyme that unwinds chromosome strands by creating double-strand breaks (DSB) in DNA and subsequently re-ligates them, facilitating replication. Inhibitors of topoisomerase II stall this process, leaving the free DNA ends to form translocations. Exposure to naturally occurring topoisomerase II inhibitors are thought to play a role in MLL fusions, characteristic of infant acute leukemia [6]. However, a link with exposure and the majority of de novo leukemia translations has not been proven, and they are thought to arise as collateral damage from endogenous proliferative, apoptotic, or metabolic stress of fetal hematopoiesis, a highly active developmental period. Abortive apoptosis has been proposed as an initiator of chromosomal translocations [7]. It has been shown that an excess of LT-HSC (hematopoietic stem cell with long-term reconstitution potential) is generated each day over what is needed for maintenance of HSC cell numbers [8] and that apoptosis is an important regulator of those numbers [9]. Proapoptotic agents have been shown to induce cleavage in *TEL* and *AML1* genes with subsequent *TEL–AML1* formation [10] and similarly *MLL–AF9* generation [11]. These data indicate that generation of fusion genes of known pathological significance is a relatively common event but the normal execution of apoptosis will remove the overwhelming majority of cells carrying these fusion genes. Having survived apoptosis, a cell with a fusion gene will expand to form a preleukemic clone if there is a permissive environment and it gives the cell a growth advantage.

SUSCEPTIBILITY AND PREVENTION OF TRANSLOCATION FORMATION

Regardless of the mechanism of translocation formation, there is evidence that the DNA damage involved may be preventable at least in some cases. Folate supplementation in pregnancy reduces the risk of c-ALL in the child [12]. Folate is necessary for the synthesis of purine and thymidine deoxyribonucleotides, and folate deficiency disrupts this process, leading to DNA instability and double-strand breakage potentially underlying translocation formation. Polymorphisms in genes involved in folate metabolism may predispose individuals to adult ALL [13], and association has been seen with specific subtypes of childhood acute leukemia [14], but the effects are less significant in the presence of adequate folate levels [15].

SECONDARY GENETIC EVENTS NECESSARY FOR PROGRESSION TO LEUKEMIA

Translocations t(8;21) and t(12;21) have been shown to occur in the normal population (before birth) at a frequency that is 100-fold greater than the risk of developing the corresponding leukemia [16]. Together with the low concordance rate (10%) observed in c-ALL in twins [17] and data from animal models [18,19], this implies that the presence of translocations alone is not sufficient to cause leukemia. It has been proposed that constitutively activating tyrosine kinase receptor (RTK) mutations, which give cells a survival/proliferative advantage, are acquired and act cooperatively with fusion genes which dysregulate differentiation, thereby leading to transformation [20]. In support of this theory is the finding that internal tandem duplication deletion mutations in RTK FLT3 (FLT3-ITD) are seen in 30–40% of acute promyelocytic leukemia (APL) patients, suggesting cooperativity with *PML–RARA* [21]. Similarly, co-transfection of FLT3-ITD with *PML–RARA* led to an APL-like disorder in mice [22]. Furthermore, *AML1–ETO* when co-transfected in mice with *TEL–PDGFR*, which constitutively activates *TEL*, generated AML [23].

THE CORE BINDING FACTOR PATHWAY: A COMMON TARGET IN UTERO?

Clearly, not all acute leukemias fit the translocation/cooperating RTK mutation model. Mutations in *FLT3*, *c-kit*, *N-RAS*, or *K-RAS*, are seen in 50% of AML cases [24]. This, along with the fact that translocations are only seen in 50% of AML cases, suggests that either not all translocations or RTK mutations have been identified, or that mechanisms other than translocation/RTK mutation such as epigenetic changes, may underlie those AML subtypes which are poorly understood at the molecular level. Three of the rearrangements determined to have an in utero origin, t(8;21), t(12;21), and inv(16), target the core binding factor (CBF) transcriptional pathway which comprises the heterodimeric transcription factor complex AML1/CBFB, generating *AML1–ETO*, *TEL–AML1*, and *MYHII–CBFB* fusion genes, respectively. This pathway represents a central regulatory platform whose importance for normal hematopoietic cell development is cell context specific [25]. The complex is a common target of rearrangement in leukemia, involved in 25% of both AML and ALL cases. As well as direct involvement of CBF partners through translocation or mutation, key CBF downstream gene function may be targeted in AML without CBF translocation or mutation. *C/EBP*α and *PU.1*, two genes whose expression is modulated by

AML1, are commonly mutated in AML cases without translocation [26]. It is possible that the CBF pathway is very sensitive to disruption during fetal hematopoiesis and as well as apoptotically generated translocations the generation of mutations in either a CBF partner or key pathway player could occur in utero. In support of this theory, abortive apoptosis has recently been proposed to lead to the generation of point mutations and loss of heterozygosity as well as translocations [11].

IMPORTANCE OF LEUKEMIC STEM CELLS

There is evidence that deletion of *TEL* in c-ALL, is subclonal to *TEL–AML1* fusion and occurs postnatally [16,27]. In individuals with *TEL–AML1*-positive c-ALL the same genomic *TEL–AML1* breakpoint was observed at leukemia presentation and relapse but the clones were characterized by *TEL* deletions with different deletion boundaries [28]. Individuals in remission retained the presence of *AML1–ETO* in various stem and progenitor cell compartments [29], suggesting the persistence of a multipotential preleukemic stem cell. These observations fit with a theory proposing that each AML subtype is generated by a specific leukemia stem cell (LSC), which is a dysregulated version of its normal counterpart, the hematopoietic stem cell (HSC) [30]. For most AML, except APL, the only cells capable of transplanting NOD/SCID mice have a phenotype similar to HSC. An HSC may acquire the necessary mutations but not functionally manifest them until a more committed progenitor stage with a permissive environment. LSCs may therefore be a phenotypically later cell than HSC and APL may be an example of this. Alternatively, as has been recently proposed, a progenitor cell may reacquire "stemness," self-renewal capacity, which together with a mutation in a key gene in an apoptosis pathway may allow it to survive long enough to acquire additional mutations or translocations [31]. Genes such as Bmi-1, a member of the polycomb group family of genes [30], and Wnt [32], are candidates for maintaining the "stemness" of both HSC and LSC.

It is important to identify the LSC for a given leukemia and to elucidate the gene expression profiles of LSCs compared directly with their counterpart HSC by microarray. Changes at the level of mutation and epigenetics (methylation, histone modification) should also be investigated. This could help to establish candidate genes with a role in leukemogenesis and could lead to novel therapies directed toward gene products expressed in LSCs but not HSCs. Several groups have characterized gene expression pathways of different leukemia subtypes by microarray and demonstrated that expression changes in small numbers of genes could classify AML by subtype (in one case even identifying a new subtype with no previously defining phenotype or chromosomal abnormality) [33–35]. However, these studies were carried out on blast cells, a more differentiated version of the LSC with more accumulated genetic changes. It would be informative to identify LSCs in "normal" individuals to facilitate the characterization of the sequence of events leading to the formation of an LSC and the evolution of leukemic clones from pre-leukemic clones. However, working with stem cell populations is extremely challenging, as demonstrated by the recent lack of overlap in findings presented by three groups investigating gene expression by microarray in stem cell populations [36–38].

ETHICAL IMPLICATIONS

Ethical implications arise from the knowledge that "normal" individuals carry translocations and preleukemic clones at significant frequencies at birth. Should these individuals be monitored for further clonal expansion? Risk of progression to overt leukemia may be modulated by lifestyle factors. Greaves' infectious hypothesis proposes that in the absence of important infection-driven modulation of the naïve immune system in infants, subsequent infectious exposures may result in highly dysregulated responses in susceptible individuals [39]. In support of this theory are the findings that lifestyle factors, such as daycare [40], and genetic factors such as variant HLA alleles that influence the immune system [41], may reduce risk. Another ethical implication, given the presence of preleukemic clones albeit at a low population frequency, is in the use of cord blood for stem cell transplantation, whereby preleukemic stem cells may be inadvertently transplanted from donor to recipient. Further studies are required to determine the frequency of a variety of leukemic translocations in different populations to comprehensively evaluate such risk.

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