Brief report

Polymorphisms in human homeobox *HLX1* and DNA repair *RAD51* genes increase the risk of therapy-related acute myeloid leukemia

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Studies of radiation-induced acute myeloid leukemia (AML) in mice suggest that the number of target stem cells is a risk factor, and the *HLX1* homeobox gene, which is important for hematopoietic development, is a candidate gene. The distribution of the C/T-3′ untranslated region (UTR) polymorphism in *HLX1* in patients with AML and therapy-related AML (t-AML) compared with controls was therefore determined. The presence of the variant *HLX1* allele significantly increases the risk of t-AML (OR = 3.36, 95% CI, 1.65-6.84). The DNA repair gene *RAD51* (135G/C-5′ UTR) polymorphism also increases t-AML risk, and when combined analysis was performed on both *RAD51* and *HLX1* variant alleles, a synergistic 9.5-fold increase (95% CI, 2.22-40.64) in the risk of t-AML was observed. We suggest that the *HLX1* polymorphism has an effect on stem cell numbers, whereas an increased DNA repair capacity *(RAD51)* will suppress apoptosis, a genetic interaction that may increase the number of genomes at risk during cancer therapy. (Blood. 2006;108:3916-3918)

Introduction

Cancer therapy carries up to a 10% risk of a secondary therapy-related acute myeloid leukemia (t-AML).[1] AML is a clonal hematopoietic stem cell malignancy, and low-penetrance susceptibility genes within the general population contribute to the risk of t-AML. The risk of malignant transformation depends on the number of mutations required and the mutation rate, so AML and t-AML genetic association studies have focused on genes encoding proteins involved in maintaining genomic stability.[2-6]

One additional theoretical t-AML risk factor is the target cell frequency because this defines the number of genomes at risk. A mouse genetic linkage analysis of radiation-induced AML (r-AML) identified 2 r-AML low-penetrance susceptibility loci on mouse chromosomes 1 and 6.[7] The chromosome 1 susceptibility locus contains the stem cell frequency regulator 1 *(Scfr1)* locus,[8,9] which determines the frequency of mouse bone marrow stem cells. AML-resistant C57 mice have a lower frequency of stem cells than other inbred mouse strains including r-AML–susceptible mice,[7-9] so stem cell frequency may be part of the equation that defines the risk of r-AML in mice.

The *Scfr1* locus (human chromosome 1q41-42) harbors the H2.0-like homeobox *(HLX1)* gene that is essential for hematopoietic development.[10] In humans, high levels of *HLX1* mRNA are found in $CD34^+$ bone marrow cells but not granulocytes or macrophages. *HLX1* mRNA levels are further increased when bone marrow cells are stimulated to proliferate and differentiate in response to cytokines and growth factors and are particularly high in AML.[11,12] Thus, *HLX1* is implicated in immature stem/progenitor cell biology and, as a homeobox transcription factor, may be involved in establishing the frequency of stem cells during early development.

We have therefore carried out a t-AML patient genetic association study on 2 *HLX1* gene polymorphisms; one causes an amino acid change (*HLX1*:C/T, P365T; NCBI dbSNP: rs2738755) and the other lies in the 3′ untranslated region (*HLX1*-C/T,3′ UTR; NCBI dbSNP: rs2738756). Polymorphisms in the *RAD51* gene promoter (−135G/C,5′ UTR), and in the epoxide hydrolase gene *(HYL1)*, which is closely linked to *HLX1* on chromosome 1, have both been previously implicated in t-AML risk.[2,6] Because t-AML risk will be highest in individuals who inherit a number of susceptibility genes, genes that encode proteins involved in DNA repair (*XRCC2*-R118H) and folate metabolism (*MTHFR*-A667V, *MTHFR*-E1298A, and *MS*-A2756T) were also assessed.

Materials and methods

Patient samples

All blood or bone marrow samples were from white patients and age-matched controls from the same geographic community and were obtained from Nottingham City Hospital following informed consent in accordance with the Declaration of Helsinki as previously reported.[5,6]

HLX1 polymorphism analyses

A 3170-bp fragment of the *HLX1* gene (NCBI accession: NM_021958.2) was amplified by polymerase chain reaction (PCR) using forward 5′-CGCTTTAGGTCTTCCGACTG-3′ and reverse 5′-TGCTTCCGGAGAGAAGTGTT-3′ primers. γ-[^{32}P]-ATP–labeled allele-specific oligonucleotides were used to genotype *HLX1*-P365T (TGGAGCCCCCGGCTGCGGA and GGAGCCCTGGCTGCGGAT) and *HLX1*-C/T (3′ UTR; ACTAGGGCGGAGGGGATC and ACTAGGGTGGAGGGGATC; underlining indicates polymorphic nucleotide) by dot blot analysis of duplicate membranes. The *HLX1* genotypes were confirmed by direct sequencing.

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Table 1. Frequency of *HLX1*-P365T and *HLX1*-C/T (3′ UTR) polymorphisms in AML and control populations and the relative risk for AML associated with these genotypes

Genotype	Controls, no. (%)	AML, no. (%)	OR (95% CI)	*P*	t-AML, no. (%)	OR (95% CI)	*P*
***HLX1*-P365T**							
CC	75 (44)	65 (42)	1.0‡	—	14 (34)	1.0‡	—
CT	76 (45)	76 (49)	1.15 (0.72-1.83)	.57	22 (54)	1.73 (0.81-3.69)	.16
TT	19 (11)	15 (10)	0.84 (0.39-1.82)	.66	5 (12)	1.02 (0.30-3.51)	.97
CT + TT*	95 (56)	91 (58)	1.08 (0.69-1.69)	.73	27 (66)	1.56 (0.75-3.23)	.23
***HLX1*-C/T (3′ UTR)**							
CC	148 (78)	126 (76)	1.0‡		22 (52)	1.0‡	
CT	37 (20)	35 (21)	1.04 (0.61-1.77)	.90	19 (45)	3.58 (1.73-7.42)	< .001
TT	4 (2)	5 (3)	1.45 (0.38-5.57)	.59	1 (2)	1.48 (0.15-14.25)	.73
CT + TT	41 (22)	40 (24)	1.08 (0.65-1.79)	.78	20 (48)	3.36 (1.65-6.84)	< .001

The AML patient groups have been compared with the control group and adjusted for age. For *HLX1*-P365T controls, n = 170; for *HLX1*-P365T AML, n = 156; and for *HLX1*-P365T, n = 41. For *HLX1*-C/T controls, n = 189; for *HLX1*-C/T AML, n = 166; and for *HLX1*-C/T t-AML, n = 42.

*Using the χ^2 test, for controls versus AML, $P = .75$; for t-AML, $P = .50$.

†Using the χ^2 test, for controls versus AML, $P = .80$; for t-AML, $P = .002$.

‡Used as a reference group.

Results and discussion

HLX1 polymorphisms

The de novo AML, t-AML, and age-matched control DNA samples were genotyped (Table 1). The variant allele frequencies in the controls were: *HLX1*-P365T, 0.40, and *HLX1*-C/T (3′ UTR), 0.14. Both were in Hardy-Weinberg equilibrium.

The adjusted odds ratio (OR) for each genotype in the AML and t-AML patients compared with controls shows that only the *HLX1*-C/T (3′ UTR) polymorphism achieved statistical significance when the patients with t-AML were compared with the controls (Table 1). The proportion of t-AML patients heterozygous (CT) for the *HLX1*-C/T (3′ UTR) polymorphism (45%) was higher than in the control (20%) or de novo AML (21%) groups ($P < .001$). The heterozygous *HLX1*-C/T (3′ UTR) CT genotype is associated with a 3.58-fold increase in t-AML risk, with a 3.36-fold increased risk of t-AML in patients who possessed at least one polymorphic T allele. There was no risk when the homozygous variant *HLX1*-C/T (3′ UTR) TT genotype was assessed, but this may be attributed to the rarity of this genotype in the general population and the relatively small number of t-AML patients in this study.

The patients with AML and the controls were assessed for 6 other functional gene polymorphisms using established methods (*XRCC2*-R118H, *MTHFR*-A667V, *MTHFR*-E1298A, *MS*-A2756T, *HYL1*-Y113H, and *HYL1*-H139R).[2,6,13-15] The epoxide hydrolase *HYL1*-Y113H genotype frequency in the controls was not in Hardy-Weinberg equilibrium ($P = .002$), but was similar to the frequency reported by Lebailly and coworkers (also not in Hardy Weinberg equilibrium).[2] This is probably due to hidden population structures that specifically affect *HYL1*. The remaining polymorphisms were in Hardy-Weinberg equilibrium ($P > .14$).

Individually, the distributions of the polymorphisms did not exhibit statistically significant differences when comparing the AML patient and control groups ($P > .1$). In total, the AML samples used in this study have been genotyped for 15 polymorphisms in 10 genes (*XRCC1*, *XRCC3*, *XPD*, and *NQO1*[5]; *RAD51* and *XRCC3*[6]; and *XRCC2*, *HYL1*, *MTHFR*, *MS*, and *HLX1* [this study]). Only the *HLX1*-C/T (3′ UTR) and *RAD51* (−135G/C) variant alleles showed any significant association with t-AML risk, and we were unable to confirm that functional polymorphisms in the epoxide hydrolase gene are associated with the risk of AML.[5] Multiple testings have been performed on these samples, and although evidence suggests that applying the Bonferroni corrections may not be appropriate in this sort of genetic association study,[16] the *HLX1*-C/T 3′ UTR polymorphism ($P < .001$) would still be highly significant if the correction were applied.

To our knowledge, this is the first time that a gene that is implicated in target cell biology has been associated with an increased risk of leukemia. The *HLX1*-C/T (3′ UTR) polymorphism lies in the 3′ UTR region of the *HLX1* gene and further investigations are required to demonstrate that it affects gene function.[17] Alternatively, it may be associated with either an as yet unidentified functional polymorphism in the *HLX1* gene or associated with another nearby causative gene polymorphism. The region between the *HLX1*-P365T and *HLX1*-C/T (3′ UTR) polymorphisms of the *HLX1* gene has been sequenced in all the samples in this study and no additional polymorphisms were found.

Combined analysis of polymorphisms in *HLX1* and *RAD51*

The DNA repair *RAD51* gene (135G/C variant allele) polymorphism conferred a 2.66-fold (95% CI, 1.17-6.02; $P = .02$) risk of

Table 2. Logistic regression analysis on combined genotypes

Patient group	Genotype: *RAD51*-135G/C	Genotype: *HLX1*-C/T (3′ UTR)	No. of samples: Control	No. of samples: AML	OR (95% CI)	*P*
De novo AML						
1	WT	WT	106	80	1.0*	—
2	WT	V	30	22	0.90 (0.47-1.71)	.75
3	V	WT	15	16	1.42 (0.66-3.06)	.37
4	V	V	4	5	1.80 (0.47-6.97)	.39
t-AML						
1	WT	WT	106	17	1.0*	—
2	WT	V	30	11	2.31 (0.96-5.57)	.06
3	V	WT	15	3	1.26 (0.32-4.87)	.74
4	V	V	4	5	9.50 (2.22-40.64)	.002

Variant (V) genotype includes all heterozygous and variant homozygous genotypes. Wild-type (WT) genotype comprises the homozygous genotype for the most common and frequent genotype. The AML patient groups have been compared with the control group and adjusted for age.

*Used as reference group.

t-AML in these same patients and controls.[6] The *HLX1*-C/T (3′ UTR) and *RAD51*-135G/C polymorphisms were therefore analyzed by combined logistic analyses (Table 2). A strong genetic interaction between the *HLX1*-C/T (3′ UTR) and *RAD51*-135 G/C was specifically observed in t-AML patients compared with controls, with a significant 9.5-fold increase in the risk of t-AML found in individuals with at least one variant *HLX1*-C/T (3′ UTR) T and at least one variant *RAD51*-135 G allele. The substantial 9.5 OR for the combined genotype is significantly higher than the sum of the individual ORs (3.36 and 2.67), suggesting there is a synergistic rather than additive genetic interaction.

The *RAD51* polymorphism leads to enhanced promoter activity and elevated mRNA expression.[18,19] The increase in cancer risk associated with an increased DNA repair capacity is counterintuitive, but a highly efficient DNA repair system may suppress apoptosis.[6] The strong synergistic genetic interaction between *HLX1*-C/T (3′ UTR) and *RAD51*-135G/C may thus be because they both increase the number of genomes at risk by determining stem cell frequency and by indirectly suppressing target cell apoptosis in response to genotoxic insult.

It must be stressed that the number of t-AML samples assessed in this study was small and much larger cohorts of t-AML samples will be required to confirm the associations described here.

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Authorship

M.J. designed research, performed research, and wrote the manuscript; C.H.S. prepared samples, analyzed data, and wrote the manuscript; N.R. contributed samples and wrote the manuscript; and M.P. designed research and wrote the manuscript.

The authors declare no competing financial interests.

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