Short Communication

Polymorphism of the CD30 Promoter Microsatellite Repressive Element Is Associated with Development of Primary Cutaneous Lymphoproliferative Disorders

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Abstract

Lymphomatoid papulosis is a preneoplastic cutaneous lymphoproliferative disorder characterized by overexpression of CD30, a member of the tumor necrosis factor receptor superfamily. CD30 signaling is known to have an effect on the growth and survival of lymphoid cells. Therefore, we hypothesized that the development of lymphomatoid papulosis and progression to an associated neoplasm such as cutaneous and systemic anaplastic large cell lymphoma may reflect an underlying genetic defect. In this study, we determined that two allelic forms of the CD30 promoter microsatellite repressive element, designated *30M377* and *30M362*, are associated with the development of lymphomatoid papulosis and CD30+ lymphomas in lymphomatoid papulosis patients, respectively. These findings suggest that allele-specific differences in the control of CD30 transcription may determine the pathogenesis of the spectrum of CD30+ cutaneous lymphoproliferative disorders. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1322–5)

Introduction

Expression of CD30, a member of the tumor necrosis factor receptor superfamily, defines a spectrum of primary cutaneous T-cell lymphoproliferative disorders including lymphomatoid papulosis and anaplastic large cell lymphoma (1, 2). Lymphomatoid papulosis is a rare disorder characterized by clinically benign recurrent nodular or papular skin eruptions that have a histologic morphology suggestive of neoplasia (3). Three lymphomatoid papulosis subtypes, designated A, B, and C, have been identified based on histologic criteria. Lymphomatoid papulosis subtypes A and C are characterized by the presence of large CD30+ atypical blasts including mononucleated and binucleated cells resembling Hodgkin Reed-Sternberg cells. In lymphomatoid papulosis subtype A, these atypical cells are surrounded by a dense inflammatory infiltrate characteristic of Hodgkin's disease whereas in lymphomatoid papulosis C, they form large sheets or constitute more than 50% of the infiltrate, resembling anaplastic large cell lymphoma. Lymphomatoid papulosis type B is composed of small to medium-sized cerebriform cells showing epidermotropism and can resemble mycosis fungoides (4).

Recurrent episodes of lymphomatoid papulosis will typically last an average of 2 to 8 weeks, followed by spontaneous resolution of lesions in the majority of patients. Despite its indolent course, between 10% and 20% of patients suffering from lymphomatoid papulosis will eventually develop lymphoma, most commonly cutaneous anaplastic large cell lymphoma, Hodgkin's disease, or mycosis fungoides (5-9). These lymphomas can also precede or coexist with lymphomatoid papulosis and, in some cases, can progress to aggressive systemic lymphomas (10-12).

The increased risk of neoplasia in patients suffering from lymphomatoid papulosis (13) suggests that there may be an underlying genetic defect associated with the development of this disorder. Interestingly, CD30+ atypical cells in lymphomatoid papulosis and associated anaplastic large cell lymphoma have been shown to share the same *TCR* gene rearrangement (14). Moreover, CD30 ligation is associated with activation of nuclear factor κB in cell lines of cutaneous anaplastic large cell lymphoma (15). These findings indicate that lymphomatoid papulosis may represent a preneoplastic disorder of T cells characterized by dysregulated expression of CD30.

Previously, we have established that the *CD30* gene may be regulated in an allele-specific manner. We identified a microsatellite of the type $[(CCAT)_{2\text{-}12}CCACTTATGCAT]_n$ between positions −1.2 kb and −336 bp of the *CD30* promoter, which acts to repress expression of the gene at the transcriptional level (16). The high degree of polymorphism seen in populations of randomly selected individuals at the *30M* promoter microsatellite locus (17) suggests that alleles of this microsatellite have the potential to differentially regulate *CD30* expression.

In this study, we have evaluated the efficacy of utilizing the highly polymorphic microsatellite within the promoter region of the *CD30* gene as a marker to predict susceptibility to the development of lymphomatoid papulosis and associated lymphoproliferative disorders and the likelihood of progression of lymphomatoid papulosis to lymphoma.

Materials and Methods

Study Population and DNA Preparation. All participants in this study were recruited with informed consent. The patient cohort included unrelated patients with lymphomatoid

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Table 1. Number and category of patients in the lymphomatoid papulosis cohort

LyP histopathologic subtype	LyP only	LyP + ALCL	LyP + HD	LyP + MF	LyP + ALCL + MF	Total
A	15 (42.9)	2 (5.7)	2 (5.7)	1 (3.1)	2 (5.7)	22 (62.8)
B	3 (8.6)	—	—	—	—	3 (8.6)
C	5 (14.3)	2 (5.7)	—	2 (5.7)	1 (2.9)	10 (28.5)
Total	23 (65.7)	4 (11.4)	2 (5.7)	3 (8.6)	3 (8.6)	35 (100)

NOTE: Percentages are shown in brackets.
Abbreviations: LyP, lymphomatoid papulosis; HD, Hodgkin's disease; MF, mycosis fungoides; ALCL, anaplastic large cell lymphoma.

papulosis with or without associated lymphoma and unrelated patients with primary cutaneous anaplastic large cell lymphoma who attended the Cutaneous Oncology Clinic at the Beth Israel Deaconess Medical Center. The cohort comprised 32 unrelated Caucasian patients diagnosed with either lymphomatoid papulosis only or lymphomatoid papulosis concurrently existing with lymphoma and 8 unrelated Caucasian patients diagnosed with primary cutaneous anaplastic large cell lymphoma. The control population comprised 57 healthy Caucasian volunteers and Caucasian patients with nonlymphoid malignancies who attended the Oncology Clinic at Beth Israel Deaconess Medical Center. Patients and controls were also gender matched. The male/female ratio was 0.500 among the lymphomatoid papulosis/anaplastic large cell lymphoma patient cohort and 0.541 in the controls. Previous studies have also indicated that there is no age or gender predilection to the development of lymphomatoid papulosis (6, 7, 9, 10, 13). To study segregation of structural heterogeneity with the *CD30* microsatellite, we also genotyped 20 members of a four-generation Caucasian family.

A small sample of peripheral blood from all subjects was dried onto FTA Whatman filter paper (Life Technologies, Victoria, Australia). Genomic DNA was isolated from dried blood spots using the QIAamp DNA blood mini kit (Qiagen, Victoria, Australia) according to the instructions of the manufacturer.

***CD30* Microsatellite Typing.** Amplification of the proximal 350 to 400 bp *CD30* microsatellite region (designated the *30M* locus) was done as previously described except for the use of an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Victoria, Australia) compared with the earlier model used in our previous studies (17). Briefly, ~100 ng of DNA were used in 50 μL PCR reactions containing 25 pmol each of the *CD30* microsatellite primers *30MSF* (5′-ACCCATTTACCCACTCACCTGC-3′) and *30MSR* (5′-CAACTGGCCTAGGGAGACTGC-3′). The *30MSF* primer was labeled with the fluorescent dye HEX at the 5′ end. PCR mixtures also contained 0.2 mmol/L deoxynucleotide triphosphates, 1 mmol/L $MgCl_2$, 2 units of FastStart Taq DNA polymerase, 1× FastStart Taq DNA polymerase buffer, and 1× GC-rich solution. PCR conditions comprised 95°C for 4 minutes, followed by 40 cycles consisting of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and a final denaturation of 72°C for 7 minutes. Sizing of PCR products was done on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Data were analyzed using the program GeneScan Analysis 3.2.1 (Applied Biosystems). GeneScan analysis sizes products to an accuracy of ±0.5 bp. Note that due to differences in the run module between the ABI 310 previously used and the later model ABI 3100 genetic analyser used in the current study, some allele sizes differ by 1 bp compared with those previously reported (17). These results reflect differences in rounding.

Results

The 32 patients suffering from lymphomatoid papulosis were classified according to histopathologic lymphomatoid papulosis subtype (A, B, or C) and the presence or absence of lymphoma at time of diagnosis. Cases of lymphomatoid papulosis concurrently existing with neoplasia were further classified according to lymphoma type (Table 1). Lymphomatoid papulosis A was the most common subtype in this cohort (63% of cases) followed by lymphomatoid papulosis C (28% of cases) and lymphomatoid papulosis B (9% cases). Three patients presented with multiple lymphomatoid papulosis subtypes; two patients had lymphomatoid papulosis subtypes A and C and one patient had lymphomatoid papulosis subtypes A and B. Of the 32 patients with lymphomatoid papulosis, 11 were diagnosed with an associated lymphoid neoplasm (i.e., anaplastic large cell lymphoma, Hodgkin's

Table 2. Distribution of *30M* alleles in controls and patients diagnosed with cutaneous lymphoproliferative disease

Allele	Controls (N = 114)	LyP and associated lymphoma diagnosis and LyP histopathologic subtype											Primary cutaneous ALCL (N = 70)
		LyP only (N = 46)			LyP + ALCL (N = 8)		LyP + HD (N = 4)	LyP + MF (N = 6)		LyP + ALCL + MF (N = 6)		Total (N = 16)	
		A	B	C	A	C	A	A	C	A	C		
30M327	1 (0.9)	—	—	—	—	—	—	—	—	—	—	0	0
30M339	3 (2.6)	—	—	—	—	—	—	—	—	—	—	0	1 (6.3)
30M342	2 (1.8)	—	—	—	—	—	—	—	—	—	—	0	2 (12.5)
30M346	9 (7.9)	2 (2.9)	—	2 (2.9)	—	1 (1.4)	—	—	1 (1.4)	—	—	6 (8.6)	3 (18.8)
30M350	2 (1.8)	—	—	—	—	—	—	—	—	—	—	0	0
30M354	1 (0.9)	—	—	—	—	—	—	—	—	—	—	0	0
30M358	17 (14.9)	5 (7.1)	1 (1.4)	2 (2.9)	1 (1.4)	1 (1.4)	1 (1.4)	—	1 (1.4)	1 (1.4)	—	13 (18.6)	3 (18.8)
30M362	1 (0.9)	—	—	—	—	—	1 (1.4)	—	—	—	—	1 (1.4)	2 (12.5)
30M365	1 (0.9)	2 (2.9)	—	—	—	—	—	—	—	—	—	2 (2.9)	0
30M369	29 (25)	6 (7.4)	1 (1.4)	3 (5.7)	—	1 (1.4)	1 (1.4)	—	1 (1.4)	1 (1.4)	—	14 (20)	2 (12.5)
30M373	39 (34)	8 (8.6)	2 (2.9)	3 (4.3)	2 (2.9)	—	1 (1.4)	1 (2.9)	1 (1.4)	1 (1.4)	1 (1.4)	20 (28.6)	3 (18.8)
30M377	9 (7.9)	7 (10)	2 (2.9)	—	—	1 (1.4)	—	1 (1.4)	—	1 (1.4)	1 (1.4)	13 (18.6)	0
30M381	0	—	—	—	1 (1.4)	—	—	—	—	—	—	1 (1.4)	0

NOTE: N refers to the total number of alleles. Percentages are shown in brackets. Zeros represent sampling errors.

Table 3. Statistical tests at the *30M* locus

Allele	Hardy-Weinberg equilibrium test (control population)				Comparison of allele frequencies in patients and controls (Fisher's exact test)		
	Expected number of alleles	Observed number of alleles	χ^2	*P*	LyP (*N* = 70) vs controls (*N* = 114)	Lymphoma-associated LyP (*N* = 24) vs controls (*N* = 114)	ALCL (*N* = 16) vs controls (*N* = 114)
					P	*P*	*P*
30M327	1.022	1	0.000	>0.05	1.000	1.000	1.000
30M339	2.930	3	0.003	>0.05	0.289	1.000	0.413
30M342	2.321	2	0.039	>0.05	0.526	1.000	0.074
30M346	8.659	9	0.010	>0.05	1.000	1.000	0.168
30M350	2.033	2	0.000	>0.05	0.526	1.000	1.000
30M354	1.022	1	0.000	>0.05	1.000	1.000	1.000
30M358	15.738	17	0.108	>0.05	0.542	0.539	0.713
30M362	0.959	1	0.002	>0.05	1.000	0.319	0.040
30M365	0.959	1	0.002	>0.05	0.559	1.000	1.000
30M369	25.309	29	0.541	>0.05	0.474	0.439	0.356
30M373	32.361	39	1.344	>0.05	0.516	0.812	0.265
30M377	8.659	9	0.013	>0.05	0.037	0.241	0.600
30M381	—	—	—	—	0.380	0.180	—

disease, or mycosis fungoides). Three of these patients were determined to suffer both anaplastic large cell lymphoma and mycosis fungoides. One patient in the cohort was diagnosed with lymphomatoid papulosis types A and C as well as anaplastic large cell lymphoma and mycosis fungoides. The patient cohort also included eight subjects diagnosed with primary cutaneous anaplastic large cell lymphoma.

Genotyping of patients and controls identified 13 *30M* promoter microsatellite alleles ranging in size from 342 to 381 bp. All alleles except *30M339* and *30M365* were consistent with the gain or loss of one or more tetranucleotide repeat units (CCAT). The most common alleles among the controls were *30M373* with a frequency of 34% and *30M369* with a frequency of 25% (Table 2).

Analysis of Hardy-Weinberg equilibrium at the *30M* locus in the control population was done using the χ^2 goodness-of-fit test. Calculated χ^2 values derived from the observed and expected numbers of each allele (Table 3) were compared with the theoretical χ^2 value with 1 degree of freedom at the 95% confidence interval, equal to 3.841. Because the calculated χ^2 values were less than 3.841 (Table 3), the null hypothesis that the population is in Hardy-Weinberg equilibrium was accepted.

To confirm that the *30M* microsatellite alleles were heritable, we genotyped 20 members of a four-generation Caucasian family. As shown in Fig. 1, we identified segregation of alleles at the *30M* locus, confirming that these structural variations in the length of the *CD30* microsatellite are not the result of somatic mutations.

Comparison of allele frequencies between the control population and patients diagnosed with lymphomatoid papulosis (Table 2) revealed a statistically significant increase in the frequency of the *30M377* allele in the patient cohort (Fisher's exact test, $P = 0.037$), suggesting that this allele may influence susceptibility to the development of lymphomatoid papulosis. There was no significant difference in the frequency of the *30M377* allele or the *30M346*, *30M358*, and *30M369* alleles and the development of a particular histologic subtype of lymphomatoid papulosis (Fisher's exact test, $P > 0.050$).

Allele frequencies were also compared between controls and patients diagnosed with both lymphomatoid papulosis and lymphoma including anaplastic large cell lymphoma, Hodgkin's disease, and mycosis fungoides. There was no significant difference in the frequency of any of the alleles between these groups (Fisher's exact test, $P > 0.050$), suggesting that the size of the *CD30* promoter microsatellite does not mark progression from lymphomatoid papulosis to lymphoma in general. However, comparison of allele frequencies between the control population and patients diagnosed with primary cutaneous anaplastic large cell lymphoma revealed a statistically significant increase in the frequency of the *30M362* allele in the anaplastic large cell lymphoma cases (Fisher's exact test, $P = 0.040$). This allele was identified in two patients who are

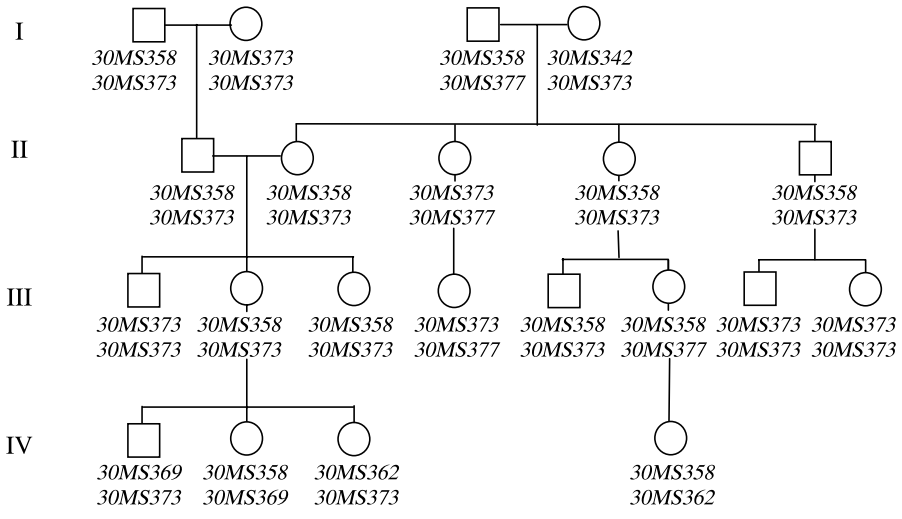

Figure 1. Pedigree illustrating segregation of *CD30* microsatellite alleles *30MS358*, *30M373*, *30MS377*, and *30M342* in members of a four-generation Caucasian family.

heterozygous at this locus. In addition, the *30M362* allele was also identified in one patient with both lymphomatoid papulosis and Hodgkin's disease. These findings suggest that allele-specific differences in *CD30* expression may affect the propensity to develop lymphomas characterized by dysregulated overexpression of *CD30*. In keeping with this view, the frequency of the *30M362* allele was found to be significantly increased in patients diagnosed with both lymphomatoid papulosis and CD30+ lymphomas (anaplastic large cell lymphoma or Hodgkin's disease) compared with controls (Fisher's exact test, $P = 0.038$).

Discussion

CD30 expression characterizes a spectrum of slowly progressive cutaneous lymphoproliferative disorders. At one end of this spectrum is lymphomatoid papulosis, a recurrent skin eruption with variable clinical outcome. Lymphomatoid papulosis lesions can undergo spontaneous regression or progress to lymphoma and metastasize to regional lymph nodes. The cause of lymphomatoid papulosis is unknown and the potential for clinical progression of this disorder cannot be predicted based on histopathologic criteria as these lesions histologically appear neoplastic in all cases (1-12). It would therefore be useful to identify susceptibility and prognostic criteria for this disorder based on the genotypic and molecular characteristics of the atypical cells.

In this study, we have tested the hypothesis that genetically controlled structural heterogeneity within the *CD30* gene could determine susceptibility to the development of lymphomatoid papulosis and influence the progression of lymphomatoid papulosis to lymphoma. We have identified two alleles at the *30M CD30* promoter microsatellite locus, designated *30M362* and *30M377*, which are present at higher frequencies in patients with lymphomatoid papulosis and related cutaneous lymphoproliferative disorders. Based on these results, a larger multicenter screening study is warranted.

The *30M362* and *30M377* alleles may mark different *CD30* haplotypes that predispose to the development of lymphomatoid papulosis. We found that the *30M377* allele is associated with the development of lymphomatoid papulosis but not with the progression of lymphomatoid papulosis to lymphoma. The type of lymphomatoid papulosis lesion marked by the *30M377* allele seems likely to undergo spontaneous regression rather than progression. On the other hand, the *30M362* allele, which is present at higher frequencies in patients with lymphomatoid papulosis and associated CD30+ lymphoma and in patients with anaplastic large cell lymphoma, may mark susceptibility for development of lesions prone to a more aggressive clinical course. Prognostic variation in the lymphomatoid papulosis disorders associated with the *30M377* and *30M362* alleles may reflect allelic differences in the control of expression of the CD30 receptor and hence its ability to signal.

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