Allelic Loss in the Progression of Myelodysplastic Syndrome

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ABSTRACT

To elucidate the genetic events that may play an important role in the progression of myelodysplastic syndrome (MDS) to acute myeloid leukemia (AML), we performed allelotyping of 24 individuals using matched MDS and AML samples from the same patients. Because the evolution can take years to occur, we used DNAs extracted from archival samples. These samples were analyzed with 79 microsatellite markers, which mapped to each of the autosomal arms except the short arms of the acrocentric chromosomes. Loss of heterozygosity on at least one locus was observed in 18 of the 24 cases (75%) as the disease progressed. Frequent allelic loss in >20% of the informative cases was observed on chromosome arms 6q (31%), 7p (23%), 10p (31%), 11q (27%), 14q (25%), and 20q (23%). Although cytogenetic information was available for many of our cases with allelic loss on 6q, 7p, 10p, 11q, 14q, and 20q, no deletions were observed on these arms. Fractional allelic loss, calculated for each sample as the total number of chromosomal arms lost per total number of arms with information, showed a median value of 0.06 and a mean of 0.15 (range, 0–0.59). No microsatellite instability at more than one marker was found in any of the samples. These results suggest that tumor suppressor genes exist on 6q, 7p, 10p, 11q, 14q, and 20q that have an important role in the evolution of MDS to AML when they are mutated.

INTRODUCTION

MDS5 comprises a heterogeneous group of hematological disorders, several of which have a high frequency of progression to AML (1). Patients with MDS frequently exhibit progressive cytopenias and increases in blast counts in peripheral blood and/or bone marrow, showing a transition to more advanced MDS subtypes with eventual development of AML.

Several lines of evidence have suggested that activation of oncogenes and inactivation of tumor suppressor genes play critical roles in the genesis and progression of neoplasms. Previous studies have noted mutations of the N-RAS and FMS oncoproteins in MDS (2–6). Although mutations of the P53 tumor suppressor gene have also been reported, little is known regarding alterations of other tumor suppressor genes in MDS (7–9). The paradigm developed by Knudson (10) is that one allele of a tumor suppressor gene is mutated and the other allele is lost through a variety of potential mechanisms, including visible chromosomal deletions. Cytogenetic studies have shown that monosomy 5, del(5q), and monosomy 7 are frequent changes, but translocations and gene amplification, as characterized by homogenous staining of the P53 RAS genes and inactivation of tumor suppressor genes play critical roles in development of AML.

deletions are assumed to show the region where a candidate tumor suppressor gene resides. However, cytogenetic studies would not reveal either mitotic nondisjunction with duplication or mitotic recombination. In addition, small deletions that are below the limits of resolution of cytogenetic analysis would not be detected.

Microsatellites are short tracts of (C-A)n repeats that exist throughout the genome and are highly polymorphic (12). Allelotyping, an extensive survey of allelic loss throughout the genome, is a tool of screening multiple loci for possibly affected tumor suppressor genes (13–25). However, these analyses in the progression of MDS are difficult to perform because paired MDS and AML samples from the same individual are required and the evolution to AML can take many years. To overcome this problem, many of our paired samples relied on DNAs extracted from archival slides of bone marrow smears obtained during the MDS and AML phases of the disease. To elucidate the genetic events that may play important roles in the progression of MDS, we performed allelotyping analysis for 24 MDS patients during their transformation to AML.

MATERIALS AND METHODS

Samples. Paired samples of bone marrow of MDS and AML after evolution from MDS were obtained from 24 patients with MDS. The 24 MDS samples consisted of 3 RA, 18 RAEB, and 3 RAEB-t. MDS was classified according to the French-American-British classification. Clinical information was available for all of the patients as shown in Table 1. The mean percentage of blasts from the bone marrow for the AML samples was 54% (range, 30–93%). Cytogenetic data were available from 22 patients. Twenty metaphases were evaluated in most samples.

Extraction of DNA. DNA was extracted from stained slides of bone marrow smears of each of the individuals (24). In addition, DNA was extracted from bone marrow mononuclear cells from three patients after obtaining their informed consent (samples 51–53). The mononuclear cells were obtained by Ficoll Hypaque gradient centrifugation.

LOH Analysis. PCR amplification of microsatellite sequences was used to determine LOH. Primers for these microsatellite sequences were obtained from Research Genetics (Huntsville, AL; Ref. 26). The loci analyzed were as follows: 1p, D1S253 and D1S46; 1q, D1S196 and D1S202; 2p, D2S146 and D2S207; 2q, D2S122 and D2S125; 3p, D3S1266 and D3S1285; 3q, D3S1272 and D3S1278; 4p, D4S418 and D4S419; 4q, D4S416 and D4S429; 5p, D5S416 and D5S418; 5q, APC and D5S107; 6p, D6S260 and D6S265; 6q, D6S283 and D6S292; 7p, D7S517 and D7S531; 7q, D7S486 and D7S487; 8p, D8S262 and D8S265; 8q, D8S272 and D8S286; 9p, D9S156 and D9S157; 9q, D9S154 and D9S176; 10p, D10S191 and D10S179; 10q, D10S190 and D10S201; 11p, D11S904 and D11S907; 11q, D11S906 and D11S923; 12p, D12S91 and D12S99; 12q, D12S96 and D12S354; 13q, D13S156 and D13S164; 14q, D14S61 and D14S70; 15q, D15S114 and D15S165; 16p, D16S404 and D16S410; 16q, D16S402, D16S411, and D16S514; 17p, D17S261 and D17S486; 17q, D17S802 and D17S805; 18p, D18S54 and D18S452; 18q, D18S60 and D18S87; 19p, D19S209 and D19S221; 19q, D19S208 and D19S214; 20p, D20S98 and D20S105; 20q, D20S100 and D20S108; 21q, D21S259 and D21S1890; and 22q, D22S282 and D22S283.

Each PCR reaction contained 25 ng of DNA, 10 pmol of each primer, 2 mol of each dNTP (Pharmacia, Stockholm, Sweden), 0.5 units of Taq DNA polymerase (Boehringer-Mannheim, Indianapolis, IN), and 3 μCi of [γ-32P]dCTP (ICN, Irvine, CA) in 20 μl of the specified buffer with 1.5 mM MgCl₂. Thirty-two cycles of amplification, PAGE, and subsequent autoradiography were performed as published previously (24). LOH was scored in

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The abbreviations used are: MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; RA, refractory anemia; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation; LOH, loss of heterozygosity; FAL, fractional allelic loss; MSI, microsatellite instability.

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informative cases if a significant reduction (>50%) in the signal of the allele from the AML phase sample was noted in comparison with the corresponding allele in the adjacent lane from the MDS sample of the same individual. In almost all samples showing LOH, PCR amplification and analysis were repeated to assure consistency of results. We performed duplex PCR to compare the intensity of two loci.

RESULTS

We screened 24 paired MDS and AML samples from the same individuals for LOH with a panel of 79 highly informative microsatellite markers representing every autosomal chromosome. Fig. 1 shows the frequency of LOH at each arm. Each sample was analyzed at one or more loci per arm except the short arms of the acrocentric chromosomes. Some samples were not examined at other loci on several arms because of a shortage of DNAs. Thirty-five of 39 chromosomal arms showed LOH for at least one matched sample (90%). LOH at one or more loci was observed in 18 of the 24 cases (75%; Table 1).

The most frequent allelic loss was observed on 6q and 10p in the AML samples (31%). Frequent allelic loss in 20% of informative cases was also observed on chromosome arms 7p (23%), 11q (27%), 14q (25%), and 20q (23%; Fig. 1). By contrast, LOH was infrequent on chromosomal arm 17p, where the P53 tumor suppressor gene resides. Representative examples of autoradiograms of LOH on chromosome arms 6q, 10p, and 20q are shown in Fig. 2. Although cytogenetic information was available for many of our cases with LOH on 6q, 7p, 10p, 11q, 14q, and 20q, no deletions were observed on these arms.

FAL was calculated for each sample as the total number of chromosome arms lost per total number of arms with information. The median FAL value was 0.06, and the mean was 0.15 (range, 0–0.59; Table 1).

We also analyzed for variations in the number of repetitive unit sequences in each microsatellite, the so-called MSI. In the present study, we did not find MSI at more than one marker in any of the AML samples.

DISCUSSION

When MDS is diagnosed, the abnormal clone is established and usually predominates in the bone marrow. Evolution to AML is considered to be associated with additional genetic changes. This
study sought to identify the number and sites of these additional alterations. As a powerful approach to this analysis, we performed allelotyping using microsatellite markers. To our knowledge, this is the first report of allelotype analysis in the progression of MDS. The most frequent allelic loss was observed on chromosome arms 6q and 10p (31%). Frequent LOH on 6q has been reported in melanoma, ovarian carcinoma, and malignant lymphoma (27–29). LOH on 10p has often been found in prostate carcinoma (30).

LOH on chromosome arm 20q was also frequent in our study (23%). Deletions or LOH on this chromosomal arm have been reported in hematological neoplasms, including polycythemia rubra vera, myelofibrosis, essential thrombocythemia, MDS, and AML (31, 32). However, to date no tumor suppressor gene has been identified on chromosome arm 20q. Further studies will be required to determine whether a single gene on 20q can mediate the evolution to AML after MDS and the above-mentioned myeloproliferative disorders.

Several of the matched MDS and AML samples from the same individual had cytogenetic abnormalities [e.g., del(5)(q?)] that were not detected by allelotyping. This can be explained because the same abnormality was present in both samples, and the contaminating normal cells, such as lymphocytes, in each sample obscured the LOH. A somewhat similar phenomenon was observed in homozygous deletion of P16INK4A in several cancers, including acute lymphoblastic leukemia (22). In these cases, the LOH at this locus could not be identified because normal cells contaminated the tumor cells.

A number of samples had LOH at certain chromosome arms but did not have a corresponding chromosomal deletion in the present study. These included chromosomes 6 and 10, which rarely are karyotypically abnormal, and chromosomes 5, 7, 11, and 20, which frequently are deleted in MDS and AML (11). Our data suggest that either mitotic nondisjunction with duplication or mitotic recombination was the possible mechanism that resulted in LOH on these chromosomes in AML. In addition, small cytogenetically undetectable deletions may be present on the chromosomal arms.

The median FAL value was 0.06 in our study. This value is lower than those reported for many solid tumors, including osteosarcoma (0.32), non-small cell lung and colorectal carcinomas (0.20), pancreatic adenocarcinoma (0.18), and bladder cancer (0.11; Refs. 13–15, 20, 21). We previously performed allelotype analysis and calculated FAL for acute lymphoblastic leukemia (0.04), chronic myelocytic leukemia evolving to blast crisis (0.06), and adult T-cell leukemia (0.09; Refs. 22, 24, 25). Taken together, allelic deletions may be less common in hematological malignancies than in solid tumors. In addition, overestimation of FAL could occur if the malignant clone was established but not predominant at initial sampling of the individual with MDS and if AML represented the marked expansion of this abnormal clone. The abnormal clone may be detectable only by our techniques. In this case, FAL occurring during the progression to AML may be an overestimate.

A prior study suggested that evolution of MDS to AML in therapy-related leukemia is associated with MSI (33). Other cancers (e.g., colon cancer) have been associated with alterations of mismatch DNA repair genes, including hMSH2, hMLH1, hPMS1, and hPMS2, and concomitant MSI (34). In this study, we do not know who or whether any of the patients had a prior exposure to a mutagen, but none of these individuals had MSI; thus, this is an unlikely cause of MDS evolving to AML.

In conclusion, this study implicates several chromosome arms as having altered tumor suppressor genes that are important in the progression of MDS to AML. Further deletion mapping of affected chromosomal regions should eventually help clone candidate tumor suppressor genes that are altered during the progression of MDS to AML.

REFERENCES


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