Short Communication

Concordance of Pharmacogenetic Polymorphisms in Tumor and Germ Line DNA in Adult Patients with Acute Myeloid Leukemia

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Abstract

Archived tumor tissue is a useful resource for retrospective studies addressing relationships between genetic polymorphisms and treatment outcomes. However, genotypes determined in tumor and somatic tissues may differ due to cytogenetic and molecular changes associated with malignant transformation and progression. Discordance between germ line and tumor genotypes may be particularly relevant in leukemia because cytogenetic abnormalities are frequent. We compared genotypes determined in DNA extracted from paired pretreatment bone marrow and buccal samples from 80 adult patients with acute myeloid leukemia (AML). Paired AML and buccal DNA samples were genotyped for polymorphisms (21 single nucleotide polymorphisms and 2 gene deletions) on genes encoding proteins involved in drug metabolism (*CYP3A4, CYP2C8, CDA*, and *GSTP1*), oxidative stress mechanisms (*CAT, MnSOD, GSTT1, GSTM1, GSTA1*, and *GPX1*), drug transport (*MDR1, MRP1*, and *BCRP*), and DNA repair (*MGMT, XPD*, and *XRCC1*). Genotypes were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, except *GSTM1* and *GSTT1*, for which deletion genotypes were determined using multiplex PCR. Concordance of genotypes was tested by κ statistics. κ statistics for paired AML and buccal DNA samples ranged between 0.94 and 1.00, indicating excellent agreement. The *GSTT1* and *GSTM1* genotypes were in perfect concordance for the paired samples. Agreement was also excellent for genes at AML chromosome deletion and translocation breakpoints, including *MDR1* at 7q21.1 and *MRP1* at 16p13.1. Based on these data, genotypes derived from archived AML bone marrow samples were not likely to differ from those from genomic DNA, and archived bone marrow samples may be useful for the conduct of retrospective pharmacogenetic studies. (Cancer Epidemiol Biomarkers Prev 2007;16(5):1038–41)

Introduction

A number of cooperative groups and academic institutions maintain archives of tumor tissues, with corresponding pathology, treatment, and clinical outcome information. These archives have enormous value in studying the relationships between molecular markers and both general prognosis and response to specific therapies, but it is unclear if genotypes derived from diseased tissue produce the same results as those from normal, non–diseased tissue. This information is of great importance for molecular epidemiologic and pharmacogenetic investigations that use archived tumor tissue as the basis for measuring genetic polymorphisms in large populations because it is assumed that these polymorphisms reflect inherited variants, rather than tumor-specific genetic changes.

Leukemia cells frequently have cytogenetic abnormalities (1-3); thus, discordance between host and tumor DNA genotypes may be of particular relevance for pharmacogenetic studies on leukemia. Genes with inherited polymorphic deletions may be of particular importance because there is great potential for misclassification if the absence of alleles is not inherited but, rather, is a result of disease-related loss of heterozygosity in tumor tissue. To determine the feasibility of using archived acute myeloid leukemia (AML) cell samples as a source of genomic DNA in future AML molecular epidemiologic studies, we conducted a methodologic study to investigate the concordance between genotypes from paired pretreatment bone marrow samples and non–diseased tissue (buccal cell samples) from 80 adult patients diagnosed with AML. Samples were genotyped for a panel of polymorphisms (21 single nucleotide polymorphisms and 2 polymorphic gene deletions) of genes encoding proteins involved in drug metabolism, protection from oxidative stress, drug transport, and DNA repair.

Materials and Methods

Study Population. The study population consisted of patients with newly diagnosed AML (ages 20-85), other than acute promyelocytic leukemia, using French-American-British classification criteria (4) at Roswell Park Cancer Institute (Buffalo, NY) between 2004 and 2006. Patients with secondary AML, i.e., with an antecedent hematologic disorder or prior chemotherapy or radiotherapy for indications other than AML were included. Patients selected for this analysis ($n = 80$) met the eligibility criteria, had cryopreserved marrow ($n = 72$) or blood ($n = 8$) AML cells available in the Roswell Park Cancer Institute Leukemia Tissue Bank and also had buccal cell samples collected for study. The study was approved by the Roswell Park Cancer Institute Institutional Review Board.

AML Samples. Bone marrow samples were aspirated into heparinized syringes and transferred to green-top tubes containing heparin and blood samples were aspirated into green-top tubes for centrifugation and processing. Most samples contained a high percentage of blasts (range, 11-99%; median, 63%), and they were further enriched for blasts by density centrifugation prior to cryopreservation. Marrow and blood samples were centrifuged over Ficoll-Hypaque, and the mononuclear cells were then aspirated, washed twice, counted, resuspended at a density of 1 to 2×10^7/mL in FCS with 20% DMSO, aliquotted into cryovials, and cryopreserved at −120°C.

Buccal Cell Samples. Buccal specimens were obtained by the swish and spit method (5). Patients were instructed to pour 10 mL of Scope (premeasured) from a specimen jar into their mouths, swish the mouthwash around their mouths vigorously for 60 s, and then spit the mouthwash back into the specimen jar and seal the lid tightly.

DNA Extraction and Amplification. DNA extraction from both buccal and bone marrow samples was done using Puregene DNA extraction kits, according to the specifications of the manufacturer.

Genotypes other than *GSTT1* and *GSTM1* were determined using Sequenom's high-throughput matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (6). Controls for genotype and two non–template controls were included on each plate, as well as 10% duplicate samples. Briefly, PCR amplification of the 80 AML and buccal DNA samples was done using single nucleotide polymorphism–specific primers, followed by a base extension reaction using iPLEX chemistry (Sequenom). The PCR condition was 94°C for 15 min for hot start, followed by denaturation at 94°C for 20 s, annealing at 56°C for 30 s, extension at 72°C for 1 min for 45 cycles, and final incubation at 72°C for 3 min. The PCR products were then treated with 2 μL of shrimp alkaline phosphatase (Sequenom) for 20 min at 37°C, then ramped to 85°C for 5 min to remove excess deoxynucleotide triphosphates, as described (7).

GSTT1 and *GSTM1* genotyping for gene deletions was carried out by a multiplex PCR as previously described (8). Primer pairs were 5′-TTCCTTACTGGTCCTCACATCTC-3 and 5′-TCACCGGATCATGGCCAGCA-3′ for *GSTT1*, which produced a 489-bp product, and 5′-GAACTCCCTGAAAAGCTAAAGC-3′ and 5′-GTTGGGCTCAAATATACGGTGG-3′ for *GSTM1*, which produced a 215-bp product. Amplification of the albumin gene (5′-GCCCTCTGCTAACAAGTCCTAC-3′ and 5′-GCCCTAAAAAGAAAATCGCCAATC-3′) was used as an internal control and produced a 351-bp product. Thirty cycles of amplification were done at 94°C for 30 s (denaturation), 64°C for 30 s (annealing), and 72°C for 30 s (extension).

Statistical Analyses. Concordance of polymorphisms between buccal and bone marrow or diseased blood samples was tested by the κ statistic, which tests the extent of agreement between the two groups. κ > 0.75 indicates excellent agreement, beyond chance; κ between 0.40 and 0.75 indicates fair to good agreement; and κ < 0.40 indicates poor agreement.

Results

A total of 23 polymorphisms in 17 genes of importance in leukemia pharmacogenetics, from 11 different chromosomal regions, were assessed. κ statistics for the paired AML and buccal DNA samples ranged between 0.94 and 1.00, indicating excellent agreement between AML cell and germ line DNA [*CAT*, 1.00; *MnSOD*, 0.98; *GSTA1*, 1.00; *GSTP1*, 0.96; *GSTT1*, 1.00; *GSTM1*, 1.00; *GPX1*, 0.96; *CYP3A4*, 0.94; *CYP2C8* (A1196G), 0.96; CYP2C8 (C9792G); *CDA*, 1.00; *MDR1-03*, 1.00; *MDR1-05*, 0.96; *MDR1-24*, 0.98; *MRP1* (exon 8), 1.00; *MRP1* (exon28), 1.00; *MRP1* (exon 9), 0.98; *BCRP* (exon 5), 1.00; *BCRP* (exon2), 1.00; *MGMT*, 1.00; *XPD312*, 1.00; *XPD751*, 0.98; *XRCC1*, 1.00; Table 1]. Most patients (90%) had identical intraindividual genotypes (complete concordance). A total of 77 paired samples (97%) had zero or one genotype discrepancy in the 23 polymorphisms between the AML and buccal cell DNA. A maximum of three discrepant polymorphisms were observed in individual cases. There was no apparent bias toward a specific allele when genotype discrepancies were observed. Three of the 23 polymorphisms (13%) had two nonmatching genotype calls. The three genes with two samples showing genotype discrepancy were located on different chromosomes and thus were not a consequence of

Table 1. Gene information and κ statistics for matched AML and buccal DNA samples (N = 80)

Gene	Single nucleotide polymorphism location	Chromosome	rs nos.	κ	Asymptotic error	Confidence interval	No. of evaluable cases (%)	No. of nonmatching genotype calls
ABCB1-03	Ex27-55A>C>G>T; I1144M	7q21.1	rs1045642	1.00			77 (96.3)	0
ABCB1-05	Ex22-9A>C>G>T; A892S	7q21.1	rs2032582	0.96	0.031	0.89-1.02	76 (95.0)	2
ABCB1-24	Ex13+12 A>G; G412G	7q21.1	rs1128503	0.98	0.023	0.95-1.02	76 (95.0)	1
ABCC1	Ex8 T825C; V275V	16p13.1	rs246221	1.00			76 (95.0)	0
ABCC1	Ex28 G4002A; S1334S	16p13.1	rs2230671	1.00			78 (97.5)	0
ABCC1	Ex9 T1062C; L562L	16p13.1	rs35587	0.98	0.022	0.93-1.02	77 (96.3)	1
ABCG2	Ex5 C421A; Q141K	4q22	rs2231142	1.00			75 (93.8)	0
ABCG2	Ex2 G34A; V12M	4q22	rs2231137	1.00			75 (93.8)	0
CAT-01	−329T>C	11p13	rs1001179	1.00			78 (97.5)	0
GPX1-01	Ex1-226C>T; P200L	3p21.3	rs1050450	0.96	0.029	0.90-1.02	79 (98.8)	2
SOD2-01	Ex2+24C>T; V16A	6q25.3	rs1799725	0.98	0.020	0.94-1.02	77 (96.3)	1
GSTA1-01	−4621T>C	6p12.1	rs3957357	1.00			78 (97.5)	0
GSTP1-01	Ex5-24A>G; I105V	11q13	rs947894	0.96	0.030	0.90-1.02	78 (97.5)	2
GSTM1	Gene deletion	1p13.3		1.00			77 (96.3)	0
GSTT1	Gene deletion	22q11.23		1.00			77 (96.3)	0
CYP3A4-02	−391A>G	7q21.1	rs2740574	0.94	0.053	0.89-1.05	77 (96.3)	1
CYP2C8	A1196G; R399K	10q23.33	rs10509681	0.96	0.040	0.88-1.04	77 (96.3)	1
CYP2C8	C792G; M264I	10q23.33	rs1058930	0.96	0.041	0.88-1.04	77 (96.3)	1
MGMT-01	Ex4-13A>G; I143V	10q26	rs2308321	1.00			80 (100.0)	0
XPD312	Ex10-16G>A; D312N	19q13.3	rs1799793	1.00			73 (91.3)	0
XPD751	Ex23-61A>C; K751Q	19q13.3	rs13181	0.98	0.022	0.93-1.02	76 (95.0)	1
XRCC1	Ex10-4A>G; Q399R	19q13.2	rs25487	1.00			74 (92.5)	0
CDA	A79C; K27Q	1p36.2-p35	rs2072671	1.00			77 (96.3)	0

NOTE: Values of κ: κ > 0.75 (excellent agreement beyond chance), κ between 0.40 and 0.75 (fair to good agreement), κ < 0.40 (poor agreement).

linked loci. Overall, there were 12 discordant genotypes among the 1,765 evaluable comparisons (0.05%). Five of the eight samples with a discrepancy were from bone marrow, whereas three were from peripheral blood.

Discussion

We showed the excellent agreement between the genotypes of 17 genes encoding proteins involved in drug metabolism, protection from oxidative stress, drug transport, and DNA repair processes, determined in 80 paired AML cells and somatic cells. Of note, the *GSTT1* and *GSTM1* gene deletion genotypes were in perfect concordance for the paired samples. These genes are of particular importance because there is great potential for misclassification if the null genotype is a result of disease-related loss of heterozygosity. We also observed excellent agreement for those genes on chromosomes that are commonly involved in deletions or translocations as part of the leukemic process, including *MDR1 (ABCB1)* at 7q21.1 and *MRP1 (ABCC1)* at 16p13.1. AML cells with monosomy 7 or del(7q), which are recurring cytogenetic abnormalities in this disease (2), are hemizygous for *ABCB1*, and *ABCC1* was found to be deleted in AML cells from 5 of 13 patients with AML with inv(16), another recurring cytogenetic abnormality in AML (9). These data show that archived bone marrow samples may be used to accurately perform genotyping for the polymorphisms that we have examined.

The discordances observed could be due to poor sample condition or to misclassification of the genotype by the Sequenom computer program. The small amount of misclassification that might occur due to these phenomena can be addressed by increasing the sample size in pharmacogenetic studies and by using statistical modeling techniques. In addition, although the data validate the use of AML samples for this panel of genotypes for AML pharmacogenetic studies, it may be important to account for gene amplification or deletion, and chromosome gain or loss when using AML cell DNA to study variation and clinical outcomes in order to account for quantitative differences that may affect the concordance between germ line genotype and cancer cell phenotype (10-12). It is possible that the discordance observed is a result of the acquisition of additional chromosomes or gene amplification that the matrix-assisted laser desorption/ionization time-of-flight genotyping methodology would not have accounted for. A study by Cheng et al. (12) suggests that amplification of specific genes or chromosomes in the cancer genome can have functional consequences that have pharmacologic relevance. It is likely that in the future, a three-dimensional strategy will need to be adopted that incorporates copy number changes with information on single nucleotide variation and clinical outcomes (10).

Only a few studies have compared polymorphisms in paired tumor and non–diseased tissue samples (13-16). In general, these investigations found a high concordance between tumor and germ line DNA samples. Stone et al. (14) reported on the degree of concordance between the *GSTM1*, *GSTP1*, and *GSTT1* polymorphisms in 78 paired normal and tumor breast tissue samples. Agreement between normal and tumor tissue was 99% for *GSTT1*, 95% for *GSTP1*, and 88% for *GSTM1*. Discordance for the *GSTM1* and *GSTT1* deletion polymorphisms was largely attributable to deletions being present in tumor tissue, but not in normal tissue. A study by Rae et al. (15) also examined genetic polymorphisms in paired tumor and blood samples obtained from 10 patients with breast cancer. The investigators specifically selected genes involved in the pharmacodynamics of chemotherapeutic drugs and observed 100% concordance between tumor tissue and blood for *CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A5*, and *MDR1*. Marsh et al. (16) compared 29 polymorphisms in 13

genes of high importance to cancer pharmacogenetics in DNA from normal mucosa and colon tumor in 44 paired samples and observed that 93% (41/44 pairs) of samples had one or zero genotype discrepancies. Seventy-seven percent of paired samples had identical tumor/germ line genotypes.

Nevertheless, results from these previous studies leave important unanswered questions. First, most studies have focused on solid tumors. Rae et al. (15) have argued that it is important to establish the utility of using diseased samples for all genetic polymorphisms that are relevant to a particular research question. Therefore, even though perfect concordance between diseased and non–diseased tissue has been established for a number of genes, it may be faulty to assume that such concordance will be present for all genes of interest in pharmacogenetic or molecular epidemiologic studies as well as in the specific tumor types of interest. It is well known that somatic mutations vary greatly among tumors, and although the likelihood that common genetic polymorphisms are targeted in tumorigenesis is low, this possibility can only be ruled out by a systematic investigation for a specific set of genes in a well-defined sample of patients with specific tumors. Genotyping of AML cells and somatic cells has only been compared in a single published study. Illmer et al. (17) did single-strand conformation polymorphism analysis in blasts and T lymphocytes isolated from 12 samples with monosomy 7 or del(7q), and found that all 12 samples showed similar single-strand conformation polymorphism patterns, i.e., hemizygosity in blasts and homozygosity in T lymphocytes.

Previous methodologic work on the utility of using archived specimens was also based on small numbers. For instance, the study by Rae et al. (15) included data from only 10 patients. Assuming that disease-related somatic alteration in these genes of interest is a rare event, a single misclassified gene in a sample of 20 patients would result in a concordance rate of 95%, which is consistent with what has been reported as the result of both fixation and embedding (13) and the disease process (14). Garcia-Closas and colleagues (18) have shown that even small degrees of misclassification in either genetic traits or environmental factors can introduce bias, which can only be addressed by substantially increasing sample sizes.

In conclusion, leukemia pharmacogenetic studies frequently rely on archived bone marrow samples as a source of DNA to predict treatment outcomes. This study confirms the feasibility and accuracy of using AML samples as a source of DNA.

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