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Impact of Polymorphic Variations of Gemcitabine Metabolism, DNA Damage Repair, and Drug-Resistance Genes on the Effect of High-Dose Chemotherapy for Relapsed or Refractory Lymphoid Malignancies

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The goal of this study was to determine whether single nucleotide polymorphisms (SNPs) in genes involved in gemcitabine metabolism, DNA damage repair, multidrug resistance, and alkylator detoxification influence the clinical outcome of patients with refractory/relapsed lymphoid malignancies receiving high-dose gemcitabine/busulfan/melphalan (Gem/Bu/Mel) with autologous stem cell support. We evaluated 21 germline SNPs of the gemcitabine metabolism genes *CDA*, deoxycytidine kinase, and *hCNT3*; DNA damage repair genes *RECQL*, X-ray repair complementing 1, *RAD54L*, *ATM*, *ATR*, *MLH1*, *MSH2*, *MSH3*, *TREX1*, *EXO1*, and *TP73*; and multidrug-resistance genes *MRP2* and *MRP5*; as well as glutathione-S-transferase *GSTP1* in 153 patients with relapsed or refractory lymphoma or myeloma receiving Gem/Bu/Mel. We studied the association of genotypes with overall survival (OS), progression-free survival (PFS), and nonhematological grade 3 or 4 toxicity. *CDA* C111T and *TREX1* Ex14-460C>T genotypes had a significant effect on OS ($P = .007$ and $P = .005$, respectively), and *CDA* C111T, *ATR* C340T, and *EXO1* P757L genotypes were significant predictors for severe toxicity ($P = .037$, $P = .024$, and $P = .025$, respectively) in multivariable models that adjusted for clinical variables. The multi-SNP risk score analysis identified the combined genotypes of *TREX1* Ex14-460 TT and *hCNT3* Ex5 +25A>G AA as significant predictors for OS and the combination of *MRP2* Ex10 + 40GG/GA and *MLH1* IVS12-169 TT as significant predictor for PFS. Polymorphic variants of certain genes involved in gemcitabine metabolism and DNA damage repair pathways may be potential biomarkers for clinical outcome in patients with refractory/relapsed lymphoid tumors receiving Gem/Bu/Mel.

INTRODUCTION

Gemcitabine is a pyrimidine nucleoside analogue with broad antitumor activity and wide clinical use. This prodrug requires cellular uptake and intracellular phosphorylation before incorporation into DNA, which is believed to be its mechanism of cytotoxicity [1,2]. The profile of gemcitabine, with dose-dependent cytotoxicity and few nonhematologic side effects, has prompted its study at high doses with autologous stem cell support [3]. Because gemcitabine inhibits DNA damage repair [4], combinations of this agent with alkylators should have synergistic or additive antitumor activity and a favorable therapeutic index.

We have developed a new high-dose combination of infusional gemcitabine with busulfan and melphalan (Gem/Bu/Mel) for lymphoid tumors, with promising results in relapsed Hodgkin's and diffuse large B cell lymphoma (DLBCL) [5,6]. The extramedullary toxicity profile of Gem/Bu/Mel includes mucositis, skin rash, and transaminase elevation. Because these nonhematologic side effects can be severe, it would be helpful to predict their occurrence for a given patient. Unfortunately, no patient or clinical features have been associated with toxicity [5].

The cellular pharmacodynamic effect of gemcitabine depends on multiple enzymes, such as those involved in its intracellular metabolism, DNA damage repair, and multidrug-resistance mechanisms, whose activity may depend on their genetic polymorphic variants. We have

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previously identified single nucleotide polymorphisms (SNPs) of key enzymes in these pathways with a major impact on clinical outcome or toxicity on patients with pancreatic cancer undergoing gemcitabine-based chemoradiation [7-14]. In contrast, the pharmacogenomics of high-dose gemcitabine have not been adequately studied. Because the effect of gemcitabine on normal and tumor cells is greater at higher doses, it is conceivable that the impact of polymorphic genetic variation of relevant enzymes may be greater in the transplantation setting.

The electrophilic alkylators busulfan and melphalan are detoxified inside the cell by reduced glutathione. Glutathione conjugation of alkylating agents is mediated by glutathione S-transferase (GST), whose activity also depends on polymorphic variations [15-17]. GST pi 1 (GSTP1) is the most abundant GST class found in many normal cell and malignant tissues [18,19]. The *GSTP1* Ile105Val polymorphism has been associated with improved outcomes in patients with myeloma receiving high-dose melphalan [20].

We hypothesized that polymorphic variations of genes involved in gemcitabine metabolism, DNA damage repair, multidrug resistance, and glutathione detoxification correlate with the toxicity and outcome of patients with relapsed/refractory lymphoid tumors receiving Gem/Bu/Mel.

MATERIALS AND METHODS

Patient Recruitment and Data Collection

This prospective study involved patients with relapsed/refractory lymphoid malignancies, including Hodgkin lymphoma, DLBCL, and myeloma, with refractory or poor-risk features that made them eligible for clinical trials of Gem/Bu/Mel with autologous stem cell transplantation at our institution [5,6]. This laboratory study was approved by the institutional review board and all patients provided informed consent before enrollment. All patients received the same treatment doses and schema of Gem/Bu/Mel, as previously described [5]. Overall survival (OS) and progression-free survival (PFS) were calculated from the date of diagnosis to date of death and progression/death, respectively. Living patients and patients without progression at the last follow-up time were censored. Nonhematologic toxicities, including mucositis, skin rash, and transaminase elevation, were graded according to the Common Terminology Criteria for Adverse Events 3.0 [21].

DNA Extraction and Genotyping

We selected 21 SNPs of the deoxycytidine deaminase (*CDA*), deoxycytidine kinase (*dCK*), human concentrative nucleotide transporter (*hCNT3*), *RECQL*, X-ray repair complementing (*XRCC*)*1*, *RAD54L*, *ATM*, *ATM* and *Rad3*-related (*ATR*), mutL homolog (*MLH*)*1*, mutS homolog (*MSH*)*2*, *MSH3*, three prime repair exonuclease (*TREX*)*1*, exonuclease I (*EXO1*), tumor protein (*TP*)*73*, multidrug resistance–associated protein (*MRP*)*2*, *MRP5*, and *GSTP1* genes according to the following criteria: (1) minor allele frequency of the SNP >15% among Caucasians; (2) coding SNPs, including nonsynonymous or synonymous SNPs; and (3) association with cancer risk or clinical outcome in previous studies. The genes, chromosome locations, nucleotide substitutions, function (such as encoding amino acid changes), reference SNP identification numbers, and minor allele frequencies of the 21 SNPs evaluated in this study are summarized in Table 1.

DNA was extracted from peripheral blood lymphocytes in a single 10-cc blood sample of patients using Qiagen DNA isolation kits (Valencia, CA). Genotyping was performed using the Taqman 5′ nuclease assay. Primers and TaqMan MGB probes were provided by TaqMan SNP Genotyping Assay Services (Applied Biosystems, Foster City, CA). The probes were labeled with the fluorescent dye VIC or FAM (Gene Link Inc, Hawthorne, NY) for each allele at the 5′ end. Polymerase chain reaction (PCR) was performed in a 5-μL total volume consisting of TaqMan Universal PCR Master Mix, 20 ng of genomic DNA (diluted with distilled H_2O), and TaqMan SNP Genotyping Assay Mix (Applied Biosystems, Foster City, CA). Allele discrimination was accomplished by running endpoint detection using the ABI Prism 7900HT Sequence Detection System and SDS 2.3 software (Applied Biosystems). Twenty percent of the samples were analyzed in duplicate, with 100% concordance in genotype calling.

Statistical Analysis

The distribution of genotypes was tested for Hardy-Weinberg equilibrium with the goodness-of-fit chi-square test. The associations of clinical factors and genotypes with OS and PFS were evaluated using log-rank test and Kaplan-Meier methods. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using univariable or multivariate Cox proportional hazard models. The association of genotypes and severe toxicity was estimated with odds ratios (OR) using univariable or multivariate logistic regression. Multivariate analyses of OS adjusted for age, number of prior chemotherapy lines, progression, and severe toxicity in this study. The effect of genotype on severe toxicity was adjusted for age, number of prior chemotherapy lines, and progression.

We estimated the false-positive report probability (FPRP) for the observed statistically significant associations using the Wacholder method [22]. FPRP is the probability of no true association between a genetic variant and a phenotype given a statistically significant finding. It depends on the observed *P* value, on the prior probability that the association between the genetic variant and the phenotype is real, as well as on the statistical power of the test. In the current study, we set the HR and OR values of 2.0 to 4.0 as a

Table 1
Single Nucleotide Polymorphisms Evaluated

Gene	Chromosome	SNP	RS No.	Minor Allele Frequency Observed*	Minor Allele Frequency Reported†
CDA	1p36.12b	Ex4 +111C>T, T145T	1048977	.31	.28
		Ex2 −76A>C, K27Q	2072671	.30	.44
dCK	4q13.3b	IVS6 −1205C>T	4694362	.41	.45
		IVS2 +9846A>G	12648166	.43	.43
hCNT3	9q21.32c	Ex14 −69C>T, L461L	7853758	.18	.15
		Ex5 +25A>G, T89T	7867504	.44	.39
RECQL	12p12	Ex15 +159A>C	13035	.40	.28
XRCC1	19q13.2	Ex6 −22C>T, R194W	1799782	.09	.15
RAD54L	1p32	Ex18 +157C>T, A730A	1048771	.10	.15
ATM	11q22-q23	IVS22 −77C>T	664677	.43	.29
		Ex38+61A>G, D1853N	1801516	.07	.16
ATR	3q22-q24	Ex4 +340C>T, T211M	2227928	.47	.38
MLH1	3q21.3	IVS12 −169C>T	2286940	.37	.41
MSH2	2p22-p21	IVS12 −6T>C	2303428	.10	.15
MSH3	5q11-q12	Ex4 −100G>A, P231P	1805355	.14	.15
TREX1	3p21	Ex14 −460C>T	11797	.38	.43
EXO1	1q42-q43	Ex15 +59C>T, P757L	9350	.20	.18
TP73	1p36.3	Ex2 +4G>A	2273953	.23	.26
MRP2	10q24.2c	Ex10 +40G>A, V417I	2273697	.19	.25
MRP5	3q27.1b	Ex10 −2A>G, Q382Q	7636910	.36	.35
GSTP1	11q13	Ex5 −24A>G, I105V	1695	.35	.46

SNP indicates single nucleotide polymorphism; RS No., reference SNP identification number.
* The data observed in current study.
† The reported minor allele frequency was from SNP500 cancer database.