Lack of CD24 Antigen Expression in B-Lineage Acute Lymphoblastic Leukemia Is Associated With Intrinsic Radiation Resistance of Primary Clonogenic Blasts

By Fatih M. Uckun and Chang W. Song

The radiation sensitivity of primary clonogenic blasts from 27 children with immunologically classified CD2-CD5-CD7-CD19-sIg- B-lineage acute lymphoblastic leukemia (ALL) was analyzed using leukemic progenitor cell (LPC) colony assays. Radiation survival curves of primary clonogenic blasts (ie, LPC) were constructed for each patient using computer programs for the single-hit multitarget as well as the linear quadratic models of cell survival. The D0 values ranged from 49 to 891 cGy (median, 239 cGy; mean ± SE, 307 ± 44 cGy) and the α values ranged from 0.000 to 2.047 Gy⁻¹ (median, 0.156 Gy⁻¹; mean ± SE, 0.284 ± 0.078 Gy⁻¹). Patients were divided into groups according to sex, age, white blood cell count (WBC) at diagnosis, plating efficiency of primary bone marrow blasts, and immunophenotype. Patient sex, age, WBC at diagnosis, or in vitro plating efficiency was not associated with radiation resistance. Notably, freshly isolated primary clonogenic blasts from patients with a CD19-CD24-CD34- composite immunophenotype (stage I B-cell precursor [BCP]) had 2.2- fold higher D0 values (P = .005) and 3.4-fold lower α values (P = .05) than those from patients with a CD19-CD24-CD34+ (stage II BCP) or CD19-CD24-CD34- (stage III BCP) immunophenotype. The relative values for CD24- primary clonogenic blasts signify greater intrinsic radiation resistance. Furthermore, the CD19-CD24-CD34- immunophenotype had a larger radiation resistant fraction. Whereas only 60% of CD19-CD24-CD34- and 33% of CD19-CD24-CD34- cases had clonogenic blasts with α ≤ 0.2, 100% of CD19-CD24-CD34- cases had clonogenic blasts with α ≤ 0.2. Furthermore, clonogenic blasts from established CD19-CD24- B-lineage ALL cell lines were significantly more radiation resistant than CD19-CD24- B-lineage ALL cell lines. Notably, radiation doses sufficient to induce apoptosis of CD24- B-lineage ALL cells were higher than those capable of inducing apoptosis in CD24+ B-lineage ALL cells. Thus, the lack of CD24 surface antigen expression in B-lineage ALL is associated with intrinsic radiation resistance at the level of primary clonogenic blasts.

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HIGH-RISK acute lymphoblastic leukemia (ALL) patients typically face a poor outcome after conventional drug treatment protocols. Until the last decade, total body irradiation (TBI) and high-dose chemotherapy have been widely used before bone marrow transplantation (BMT) in aggressive attempts to eradicate residual disease in remission patients as a defense against relapse. Although improvements in long-term disease-free survival after BMT have been reported, recurrence of leukemia remains a major threat in high-risk ALL. Several possible factors may limit the ability of intensive pre-BMT radiation conditioning to prevent relapse. Clonogenic blasts (ie, leukemic progenitor cell [LPC]) may (1) possess an intrinsic resistance to radiation, (2) contain a large fraction of noncycling dormant blasts, (3) effectively repair sublethal radiation injury, (4) be capable of rapid self-renewal and repopulation, and/or (5) prevail simply as a result of a high leukemia burden that cannot be destroyed by current conditioning regimens.

Until recently, very little was known about the radiobiologic features of primary leukemic blasts from ALL patients. This paucity of knowledge was due to historic difficulties in cloning freshly isolated primary ALL blasts in vitro. Within the past 6 years, we have refined in vitro colony assay systems to culture primary blasts from T-lineage ALL as well as B-lineage ALL patients. These assay systems have provided evidence indicating that in vivo clonogenic ALL blasts, referred to as LPC, behave similarly to their in vivo counterparts.

The results of radiation-based pre-BMT conditioning regimens are not likely to sufficiently improve until we learn more about the effects of TBI on clonogenic blasts. A thorough radiobiologic analysis of primary clonogenic blasts from ALL patients is a requisite step toward greater understanding of clinical radiation resistance. In a recent preclinical study, the T-lineage ALL LPC colony assay system was used to compare the radiobiologic features of primary clonogenic blasts from 34 newly diagnosed T-lineage ALL patients. T-lineage ALL patients varied considerably relative to the intrinsic radiation sensitivity of their clonogenic blasts. Notably, the expression of the CD3 surface antigen appeared to be an overwhelming predictor of radiation resistance in vitro as well as in vivo.

In the present study, we evaluated the radiation sensitivity of primary clonogenic blasts from 27 newly diagnosed B-lineage ALL patients. Two computer-based models of cell survival have been designed to analyze intrinsic radiation sensitivity. The single-hit multitarget model of survival curve analysis generates D0 values and SF2 values. The D0 value is measured from the straight portion of the radiation survival curve anal....
dose survival curve and represents the dose required to reduce the clonogenic fraction of leukemic blasts by 63% (37% slope). The SF2 value is determined by the surviving fraction of clonogenic blasts at 200 cGy. Intrinsic radiation sensitivity in the linear quadratic model[21-23] is based on the α value, representing the initial slope of the linear component of the continuously bending radiation dose survival curve. High α values and low Do and SF2 values serve as positive indicators of the radiation sensitivity of clonogenic blasts.

Clonogenic blasts from different B-lineage ALL patients varied substantially in their sensitivity to radiation, as reflected by marked interpatient variation in the values obtained from survival curves of LPC. We therefore initiated a search for possible relationships between the radiation responses of clonogenic blasts and the standard diagnostic features of our patients, including age, sex, white blood cell count (WBC) at diagnosis, and immunophenotype. Our most notable observation was based on immunophenotypic data, which provided unprecedented evidence that radiation resistance in B-lineage ALL is associated with a CD19+CD24-CD34+ composite immunophenotype. To our knowledge, this report represents the first radiobiologic analysis establishing the association between a specific composite immunophenotype and intrinsic radiation resistance at the level of primary clonogenic blasts from B-lineage ALL patients.

MATERIALS AND METHODS

Patient selection and patient material. Fresh leukemic BM samples from 27 newly diagnosed B-lineage ALL patients were used to study the radiobiologic features of primary clonogenic blast populations (Table 1). Diagnosis of B-lineage ALL was based on the morphologic, cytochemical, and immunophenotypic features of marrow blasts. Fresh BM aspirates were obtained by conventional methods as part of clinical treatment protocols. Mononuclear cells highly enriched with leukemic blasts (89% ± 2% of mononuclear cells were CD19+ B-lineage ALL blasts; see Table 1) were isolated from fresh BM aspirates by density gradient separation on Ficoll-Hypaque gradients. In 23 of 27 cases, 82% ± 4% of mononuclear cells expressed the common acute lymphoblastic leukemia antigen (CALLA) CD10 (Table 1). All patient BM samples were used following the guidelines of the University of Minnesota Committee on the Use of Human Subjects in Research for secondary use of pathologic or surgical tissue. Because the performance of this study required large numbers of surplus cells, only cases with hypercellular BM aspirate samples could be included.

Cell lines. In addition to primary ALL blasts, we also used cells from the CD19+CD24+ pre-pre-B ALL cell line REH and RH (an in vivo clonogenic subclone of REH that kills SCID mice of disseminated human leukemia); the pre-B ALL cell lines (1) MO [a t(1;19) Cm+ pre-B ALL cell line], (2) MOD (an in vivo clonogenic subclone of MO that kills SCID mice of disseminated human leukemia), and (3) NALM-6 [a t(8;14) Cm+ pre-B ALL cell line]; the CD19+CD24+ B-lineage ALL cell lines KM and HB; and a CD19+CD24- subclone of the t(4;11) ALL cell line RS4;11 that was passaged in SCID mice (v12, RS4;11 SCID).

Immunophenotyping. The surface antigen profiles of freshly isolated B-lineage ALL blasts were analyzed by indirect immunofluorescence and flow cytometry using a panel of monoclonal antibodies (MoAbs) that define human leukocyte differentiation antigens, as previously described.[16-24] Specifically, we used the MoAbs 13-B3/35.1 (anti-CD2), T101/10.2 (anti-CD5), G3.7 (anti-CD7), BA-3 (anti-CD10), B43 (anti-CD19), BA-1 (anti-CD24), and MY10 (anti-CD34). Samples were considered positive if greater than 30% of cells bound the antibody used because the analyzed BM mononuclear cell preparations contained greater than 70% leukemic blasts.

Irradiation of cells. Primary B-lineage ALL blasts from freshly obtained BM aspirate samples, 1 x 10^5 cells/mL, in α-minimal essential medium (α-MEM) supplemented with 5% fetal calf serum, were irradiated with 50 to 800 cGy gamma rays in a single exposure (15 or 100 cGy/min at 37°C) using a 137Cs irradiator (Model Mark I; J. L. Shephard and Assoc, Glendale, CA), as previously described.[20,23,27] After irradiation, 1 x 10^6 ALL blasts per sample were assayed in duplicate for B-lineage ALL blast colony formation, as described below. The survival fractions of clonogenic blasts were determined using the formula: percent survival = 100 x (mean number of colonies in irradiated samples)/(mean number of colonies in nonirradiated controls).
uniradiated control samples). The radiation survival curves were constructed using computer programs for the analysis of cell survival data according to the single-hit multitarget and linear quadratic models, as detailed under data analysis below. Data were expressed as the mean ± SE values for the radiation sensitivity parameters $D_0$ value (37% dose slope) and $SF_2$ value (surviving fraction at 200 cGy) of the multitarget model and $\alpha$ value (initial slope reflecting the steepness of the linear component of cell killing) of the linear quadratic model.

Colony assays for primary clonogenic blasts from B-lineage ALL patients. Fresh ALL marrow blast samples (1 X 10^5 blasts/mL, duplicate 1 mL samples) were irradiated and immediately plated in 35-mm Petri dishes for a 7-day culture at 37°C in a humidified 5% CO₂ atmosphere in α-MEM. Medium was supplemented with 0.9% methylcellulose, 50 μmol/L 2-mercaptoethanol, 15% human plasma + 15% fetal bovine serum (or 30% calf bovine serum), and 10% (vol/vol) low molecular weight B-cell growth factor (L-BCGF; Cellular Products, Buffalo, NY).16,17,27 On day 7, blast colonies containing greater than 20 cells were counted using an inverted phase microscope with high optical resolution. Colony blasts were then subjected to morphologic and immunologic analyses, as described.28 In all experiments, culture dishes were examined immediately and 24 hours after plating to exclude the possibility of clumping not related to proliferation, which might lead to wrong conclusions regarding the radiation sensitivity of clonogenic blasts.

Colony assays for established B-lineage ALL cell lines. Cells from established B-lineage ALL cell lines (1 X 10^5 cells/mL, duplicate 1 mL samples) were irradiated and immediately plated in 35-mm Petri dishes for a 7-day culture at 37°C in a humidified 5% CO₂ atmosphere in α-MEM. Medium was supplemented with 0.9% methylcellulose, 50 μmol/L 2-mercaptoethanol, and 10% fetal bovine serum. On day 7, blast colonies containing greater than 20 cells were counted using an inverted phase microscope with high optical resolution.

Data analysis. The radiation survival curves were constructed using three Macintosh computer programs. ENTER, DSUM, and FIT (kindly provided by Dr N. Albright, Department of Radiation Oncology, University of California, San Francisco, CA) for the analysis of cell survival data according to the single-hit multitarget and linear quadratic models of cell survival.20,21 The program ENTER receives and stores data on the computer’s disk, thus assisting the user in establishing files of dose-survival data. The program DSUM reads the file created by ENTER, calculates the survival fractions, corrects these for cell multiplicity at the time of irradiation, and calculates a weight for each data point on a survival curve based on the variation of the number of surviving clonogenic units in irradiated samples, a Poisson distribution, and the variance of the data points about a fitted curve.21 The program ultimately makes a file containing radiation dose, clonogenic cell survival, standard errors of the data points, and associated weights. The program FIT reads the files made by DSUM, fits survival curves by iteratively weighted least squares to each set of dose-survival data or to the combined data from several sets of dose-survival data, estimates the covariances of the survival curve parameters and the corresponding confidence regions, plots the survival curve and data points on the user’s terminal, and makes output files containing the survival curve parameters and plotting information. FIT calculates dose-survival curves for each of several cell survival models selected by the user. The data in this study were expressed as $D_0$ value and $SF_2$ value representing the radiation sensitivity parameters of the single-hit multitarget model of cell survival, as well as the $\alpha$ value representing the radiation sensitivity parameters of the linear quadratic model of cell survival.20,21 We used standard statistical methods, including Student’s t-tests, to evaluate the in vitro radiation survival data and to compare the radiobiologic features of primary clonogenic blasts from different patients, as previously described.20,27 $X^2$ analyses were used to assess the degree of association between clinical/laboratory parameters and radiation resistance/sensitivity of LPC. All computations were performed using the updated StatWorks statistics program for the Macintosh from Cricket Software (Philadelphia, PA).

Apoptosis assays. Cells were harvested 4 hours after exposure to ionizing radiation and DNA was prepared for analysis of fragmentation, as described.29 DNA was then electrophoresed through a 1% agarose gel and visualized by UV light after staining with ethidium bromide (0.5 μg/mL). HaeIII-digested λ DNA was used for sizing, as previously reported.28

RESULTS AND DISCUSSION

Clinical, immunophenotypic, and radiobiologic features of B-lineage ALL patients. Twenty-seven children with newly diagnosed B-lineage ALL were included in this study. Patient characteristics are detailed in Table 1. There were 10 females (37%) and 17 males (63%) with a median age of 5 years (range, 1 to 17 years; mean ± SE, 6 ± 1 years). Twenty-two of 27 patients (88%) were less than 10 years of age. The initial WBC ranged from 4.0 X 10^9/L to 999.0 X 10^9/L (median, 58.0 X 10^9/L; mean ± SE, 135.2 ± 54.8 X 10^9/L). The im-

![Fig 1. Radiation sensitivity of primary clonogenic blasts from newly diagnosed B-lineage ALL patients according to plating efficiency. The radiation sensitivity (ie, $\alpha$ and $SF_2$ values of the single dose radiation survival curves) of B-lineage LPC was correlated on a continuous scale with LPC plating efficiency by simple regression. The coefficient of correlation (CC) for $\alpha$ values and plating efficiency was 0.158, with a $P$ value for statistical significance of 0.432. Similarly, the CC for $SF_2$ values and plating efficiency was 0.024, with a $P$ value of 0.306. Thus, there was no significant correlation between plating efficiency and $\alpha$ or $SF_2$ values.](www.bloodjournal.org)
Table 2. Statistical Correlations

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>N</th>
<th>(D_0) (cGy)</th>
<th>(P)</th>
<th>(\alpha) (cGy(^{-1}))</th>
<th>(P)</th>
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<tr>
<td>Female</td>
<td>10</td>
<td>230 ± 33</td>
<td>NS</td>
<td>0.199 ± 0.059</td>
<td>NS</td>
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<tr>
<td>Male</td>
<td>17</td>
<td>361 ± 65</td>
<td></td>
<td>0.335 ± 0.118</td>
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<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;5</td>
<td>13</td>
<td>346 ± 56</td>
<td>NS</td>
<td>0.163 ± 0.045</td>
<td>NS</td>
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<tr>
<td>≥5</td>
<td>14</td>
<td>277 ± 86</td>
<td></td>
<td>0.397 ± 0.139</td>
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<tr>
<td>WBC</td>
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<td></td>
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<tr>
<td>&lt;100,000/(\mu)L</td>
<td>13</td>
<td>301 ± 62</td>
<td>NS</td>
<td>0.227 ± 0.056</td>
<td>NS</td>
</tr>
<tr>
<td>≥100,000/(\mu)L</td>
<td>14</td>
<td>206 ± 43</td>
<td></td>
<td>0.184 ± 0.109</td>
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<tr>
<td>Plating efficiency</td>
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<tr>
<td>&lt;2.06%</td>
<td>13</td>
<td>224 ± 59</td>
<td>NS</td>
<td>0.342 ± 0.150</td>
<td>NS</td>
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<tr>
<td>≥2.06%</td>
<td>14</td>
<td>357 ± 62</td>
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<td>0.231 ± 0.058</td>
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<tr>
<td>CD19+CD24^-CD34^+</td>
<td>5</td>
<td>564 ± 130</td>
<td>.006</td>
<td>0.094 ± 0.030</td>
<td>.05</td>
</tr>
<tr>
<td>CD19+CD24^+CD34^+</td>
<td>10</td>
<td>248 ± 72</td>
<td>NS</td>
<td>0.256 ± 0.114</td>
<td>NS</td>
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<tr>
<td>CD19+CD24^-CD34^-</td>
<td>12</td>
<td>244 ± 25</td>
<td></td>
<td>0.317 ± 0.065</td>
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</table>

The \(D_0\) and \(\alpha\) values (mean ± SE) of primary clonogenic blasts from newly diagnosed B-lineage ALL patients were compared using two-sample, two-sided Student's \(t\)-tests. \(N\) refers to the total number of patients in each group. \(D_0\) values could be determined in 25 of 27 patients, whereas \(\alpha\) values were determined in all 27 cases.

Abbreviation: NS, not significant.

Immunophenotypic features of leukemic blasts were determined by immunofluorescence staining and flow cytometry using a broad panel of MoAbs reactive with lymphoid differentiation antigens. None of the 27 patients expressed the pan-T-cell antigen CD2, CD5, or CD7, or the mature B-cell marker surface Ig (sIg) (Table I). In contrast, leukemic blasts from each of the 27 patients expressed high levels of CD19 B-lineage differentiation antigen. Eighty-nine percent (±2%) of leukemic blasts from 27 of 27 (100%) expressed CD19, and 88% ± 2% of leukemic blasts from 22 of 27 patients (81%) expressed CD24. The lymphoid precursor cell antigen CD10 was expressed on 82% ± 4% of leukemic blasts from 23 of 27 (85%) patients. The progenitor cell associated antigen CD34 was expressed on 74% ± 5% of leukemic blasts from 15 of 27 patients (56%). These immunophenotypic features are consistent with B-lineage ALL.29
Primary BM blasts from all 27 patients were exposed to ionizing radiation, cultured in the presence of L-BCGF, and assayed for B-lineage ALL blast colony formation. The blast colony numbers ranged from 108 colonies/10^5 cells plated (0.108%) to 4,536 colonies/10^5 cells plated (4.536%) (median, 2,060 colonies/10^5 cells [2.06%]; mean ± SE, 1,911 ± 268 colonies/10^5 cells [1.911% ± 0.268%]). The morphologic and immunophenotypic features of colony blasts were consistent with B-lineage ALL (data not shown). These immunophenotypic features are consistent with B-lineage ALL and exclude the possibility of outgrowth by CD2,5,7+ T-lineage lymphoid cells or sIg+TdT- mature B-lineage lymphoid cells.

Radiation survival curves of primary clonogenic blasts were constructed for each of the 27 patients using computer programs for the single-hit multitarget as well as the linear quadratic models of cell survival. The computer-determined values representing radiobiologic parameters indicated a marked interpatient heterogeneity in intrinsic radiation sensitivity of clonogenic blasts. Notably, the α values ranged from 0.000 to 2.047 Gy⁻¹ (median, 0.156 Gy⁻¹; mean ± SE, 0.284 ± 0.078 Gy⁻¹) (Table 1).

Because of the observed variation in both the plating efficiency and the radiobiologic parameters, it was important to formally exclude the possibility that the radiation responses of B-lineage LPC were affected by their plating efficiency. As shown in Fig 1, we found no correlation between α values (P = 0.432) or SF₂ values (P = 0.906) and the plating efficiency. We next divided patients into three groups of equal size according to the LPC plating efficiency. The plating efficiency categories were less than 1% (category I, N = 9), 1% to 2.2% (category II, N = 9), and greater than 2.2% (category III, N = 9). Plating efficiency showed no significant effect on α or SF₂ values across these three categories (α values: category I v category II = 0.354 ± 0.217 v 0.286 ± 0.075, P = .769; category I v category III = 0.354 ± 0.217 v 0.213 ± 0.069, P = .544; category II v category III = 0.286 ± 0.075 v 0.213 ± 0.069, P = .490; SF₂ values: category I v category II = 0.573 ± 0.106 v 0.521 ± 0.070, P = .686; category I v category III = 0.573 ± 0.106 v 0.561 ± 0.072, P = .925; category II v category III = 0.521 ± 0.070 v 0.561 ± 0.072, P = .695).

Correlation between immunophenotype and radiation sensitivity of primary B-lineage ALL blasts. Patients were divided into groups according to sex, age, WBC at diagnosis, and immunophenotype (Table 2). Notably, freshly isolated primary clonogenic blasts from patients with a CD19*CD24*CD34* composite immunophenotype (stage I B-cell precursor/BCP) had 2.2-fold higher Do values (P = .005) and 3.4-fold lower α values (P = .05) than those from patients with a CD19*CD24*CD34* (stage II BCP) or CD19*CD24*CD34* (stage III BCP) immunophenotype. Higher Do values and lower α values indicate greater intrinsic radiation resistance. Furthermore, the CD19*CD24*CD34* immunophenotype had a larger radiation resistant fraction (Fig 2). Whereas only 60% of CD19*CD24*CD34* and 33.3% of CD19*CD24*CD34* cases had clonogenic blasts with α ≤ 0.2, 100% of CD19*CD24*CD34* cases had clonogenic blasts with α ≤ 0.2 (CD19*CD24*CD34+ v CD19*CD24*CD34-: x² = 1.34, P = .2; CD19*CD24*CD34+ v CD19*CD24*CD34-: x² = 5.1, P = .02). Thus, the lack of CD24 surface antigen expression in B-lineage ALL is associated with intrinsic radiation resistance at the level of primary clonogenic blasts.

The radiobiologic differences between LPC from CD24* patients and CD24+ patients were not due to differences in plating efficiency because the LPC plating efficiency of CD24* patients did not significantly differ from the LPC plating efficiency of CD24+ patients (1.616% ± 0.210% v 1.205% ± 0.433%, P = .413).

As shown in Fig 3, the SF₂ and α values of the composite radiation survival curve of clonogenic blasts from patients with a CD19*CD24*CD34* immunophenotype were 0.65 and 0.168 Gy⁻¹, respectively, consistent with marked radiation resistance. In contrast, the SF₂ and α values of the composite radiation survival curves for clonogenic blasts from patients with CD19*CD24*CD34* immunophenotype were 0.49 and 0.363 Gy⁻¹, respectively, consistent with significantly greater radiation sensitivity (Fig 3).

Patient sex, age, WBC at diagnosis, CD10 expression, or in vitro plating efficiency did not demonstrate an impact on the radiation sensitivity of clonogenic blasts (Table 2).
CD34+ ALL cell lines REH and MOD (Fig 4B). These results corroborate our findings regarding the association of CD24 antigen expression with radiation sensitivity of primary B-lineage ALL blasts.

Radiation-induced apoptosis in CD24+ and CD24- B-lineage ALL cells. Clonogenic assays do not permit the analysis of the radiation sensitivity of the total cell population. Therefore, we next compared the extent of radiation-induced apoptosis as a measure of radiation sensitivity in CD24+ versus CD24- B-lineage ALL cells. Whereas ionizing radiation with 100 or 200 cGy γ-rays at a dose rate of 15 cGy/min induced distinctive morphologic changes indicative of apoptosis, including nuclear chromatin condensation, segmentation of the nucleus, and plasma membrane blebs, in greater than 50% of CD24+ NALM-6, RH (a subclone of the REH cell line), or MO cells, it induced such changes in less than 10% of CD24- RS4;11 or AN cells (data not shown). DNA from CD24+ NALM-6, RH, and MO cells showed marked fragmentation even after 100 cGy γ-rays, whereas radiation doses ≥ 400 cGy were necessary to induce apoptosis in CD24- RS4;11 and AN cells (Fig 5). Notably, the extent of DNA fragmentation in CD24- ALL cells even after 800 cGy γ-rays was not as pronounced as the extent of DNA fragmentation in CD24+ ALL cells exposed to only 100 to 200 cGy (Fig 5).

In summary, we used the B-lineage ALL LPC colony assay system to measure radiobiologic parameters of primary clonogenic blasts from 27 newly diagnosed B-lineage ALL patients. Values generated by the computer-based single-hit multitarget and linear quadratic models of cell survival were then evaluated in relation to standard diagnostic features including age, sex, WBC at diagnosis, and immunophenotype. Among these diagnostic parameters, only immunophenotype showed a significant correlation with the intrinsic radiation sensitivity of clonogenic blasts.

Our findings are the first indication that lack of CD24 surface antigen expression in B-lineage ALL is associated with intrinsic radiation resistance at the level of primary clonogenic blasts. CD24 is a two-chain glycoprotein antigen that is present on the surface of B-lineage lymphoid cells. This marker is acquired at a very early developmental stage and is expressed throughout the B-cell ontogeny.39,40 Recently, a cDNA for the CD24 antigen has been cloned.41 The CD24 cDNA encodes a very short peptide of only 31 to 35 amino acids that is extensively glycosylated and attached to the plasma membrane by a glycosyl phosphatidylinositol (GPI) anchor.42 Although cross-linking of CD24 on B-lineage ALL cell lines does not generate a mitogenic or antiproliferative signal,43 agonistic anti-CD24 MoAbs trigger calcium mobilization and H₂O₂ production and also modulate the responses of B-lineage lymphoid cells to activation signals.44 The biochemical signal generated by triggering of the CD24 receptor is likely linked to an active protein tyrosine kinase (PTK) signaling pathway because the CD24 receptor has been shown to associate with PTKs that are key regulators of cell activation and signal transduction.45 Such behavior is reminiscent of the ability of several other GPI-linked cell surface molecules to complex with PTK. As a result of this physical association with PTK, CD24 antigen expression and/or its ligation with agonistic antibodies is likely to modulate the biologic outcome of other signals that are dependent on activation of PTK. Intriguingly, recent studies in our laboratory demonstrated that activation of PTK and tyrosine phosphorylation are important proximal steps in radiation-induced apoptosis and clonogenic cell death of human B-cell precursors, including B-lineage ALL blasts.46 How ionizing radiation induces activation of PTK is yet unknown. We speculate that alterations in the plasma membrane induced by ionizing radiation can trigger the CD24-linked PTK pathway, and this signal contributes to the radiation-induced death of B-lineage ALL blasts. Therefore, higher radiation doses may be required to generate an apoptotic PTK signal in CD24+ cells. Perhaps an altered radiation dose threshold for generating apoptotic signals is the underlying basis for the association observed between the CD24+ immunophenotype and radiation resistance.

D₀ value is a radiobiologic parameter representing the dose required to reduce the clonogenic fraction of leukemic blasts by 63%. The mean D₀ values for most human tumor cell lines range from 100 to 160 cGy.47 In this report, the mean D₀ value of primary LPC from CD19+CD24+CD34- B-lineage ALL patients is 554 ± 130 cGy. The α value, reflecting the initial slope of radiation survival curves constructed according to the linear quadratic model of cell survival, is one predictor of the sensitivity of human tumors to clinical radiation.48 The reported α values for human tumor cells range from 0.2 to 0.6 Gy⁻¹.22,35,45 The mean α value for LPC from CD19+CD24+CD34- patients in the present study is 0.094 ± 0.030 Gy⁻¹. Notably, 80% of CD19+CD24+CD34- patients had SF₂ values ≥ 0.50, which are equivalent to the reported SF₂ values for the least radiation-responsive tumors in clinical radiation therapy.22,49 Thus, primary clonogenic blasts from CD19+CD24+CD34- B-lineage ALL patients are clearly among the most radiation-resistant human tumor cells currently recorded.

This report extends our earlier work on the radiobiologic features of primary leukemic blasts from ALL patients20,27 and provides novel insights into possible associations between
immunophenotype and radiation sensitivity. The results of this study recommend a detailed analysis of the effects that CD24 antigen expression may have on the outcome of TBI-based BMT protocols. In view of the observed radiation resistance of B-lineage LPC and the disappointingly high relapse rate among B-lineage ALL patients undergoing BMT, we recommend that future TBI regimens for B-lineage ALL be based on the radiobiologic features of B-lineage LPC, which challenges the concepts that formed the basis for current fractionated TBI protocols. While amplifying our knowledge of the radiobiologic features of human neoplastic cells, our results encourage the development of novel agents capable of radiosensitizing B-lineage LPC that may help in the design of more effective TBI conditioning regimens in BMT for B-lineage ALL.

REFERENCES


Lack of CD24 antigen expression in B-lineage acute lymphoblastic leukemia is associated with intrinsic radiation resistance of primary clonogenic blasts

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