ABSTRACT

The blast progenitors in acute myelogenous leukemia grow in response to hematopoietic growth factors (HGFs), and their sensitivity to antileukemic drugs is influenced by HGFs. We report the effects of stem cell factor (SCF) on the growth and sensitivity to 1-β-D-arabinofuranosylcytosine (Ara-C) of blast progenitors in acute myelogenous leukemia. SCF stimulated both colony formation and self-renewal of blast progenitors and, when used in combination with other HGFs, synergistic enhancement of colony formation was noted in 8 of the 15 patients examined. Cell fractionation studies demonstrated no unique growth dependency on SCF in either CD34+ or CD34− populations. Blast cells of patients that displayed synergistic growth enhancement with SCF displayed the highest Ara-C sensitivity when HGFs were used in combination with SCF. The titrated thymidine suicide test (20-min exposure) revealed that the proportion of blast progenitors in the S phase of the cell cycle was highest when SCF and another HGF were simultaneously present, although 24-h exposure killed most or all of the blast progenitors. These data indicate that SCF enhances growth and sensitivity to Ara-C of acute myelogenous leukemia blast progenitors in a closely correlated fashion and that the cell cycle changes as well as other mechanisms are involved in the Ara-C sensitivity modulation by SCF.

INTRODUCTION

SCF, variously designated as kit ligand, mast cell growth factor, or steel factor, is a novel hematopoietic growth factor identified as a ligand to the tyrosine kinase receptor encoded by the protooncogene c-kit (1–9). SCF supports early hematopoiesis directly as well as in synergy with a variety of HGFs including IL-3, GM-CSF, G-CSF, erythropoietin, IL-1, IL-6, and IL-7 (3, 5–7, 10–13). Individual HGFs, when used alone, have only submaximal or no effect on highly enriched hematopoietic stem cells but strongly stimulate their growth and differentiation in the presence of SCF (11). It has been found that SCF administered in vivo causes bone marrow hyperplasia and leukocytosis (14) or an increase in the number of hematopoietic progenitor cells in the bone marrow and spleen (15). Thus, data from previous studies indicate that SCF plays an essential role in hematopoiesis both in vitro and in vivo, particularly at the earliest stage of stem cell differentiation.

The blast cells in AML are a functionally heterogeneous population of cells maintained by a small number of cells capable of self-renewal and differentiation called blast progenitors, the leukemic counterpart of hematopoietic stem cells (16). It is now well known that the growth of blast progenitors is regulated by physiological HGFs including GM-CSF, G-CSF, IL-3 (17–20), M-CSF (21), IL-1, IL-4 (22, 23), IL-5, IL-6 (20), and erythropoietin in a similar if not identical fashion to normal stem cells. Although we and others have found that HGFs could modify the sensitivity of blast progenitors to cytotoxic drugs including Ara-C (24–28), there has been controversy as to whether HGFs could be used safely in patients with AML (29–31) and whether such cytokine-combined chemotherapy is, in fact, effective. Based on the in vitro experimental data, clinical trials using HGFs, including GM-CSF, in conjunction with chemotherapy for patients with AML have been initiated (32–34).

This paper describes the in vitro effects of SCF on the proliferation and self-renewal of blast progenitors in AML and reports experimental data suggesting that SCF might be a useful cytokine, when used in combination with other HGFs, in intensifying the cytotoxic effects of Ara-C on blast progenitors in AML. Based on the culture data presented in the first part of this paper, patients were chosen, and the sensitivity of their blast progenitors to Ara-C was examined using suspension culture and clonogenic assay. The highest sensitization of the blast progenitors to Ara-C was achieved when cytokines were used together with SCF. The influence of HGFs, including SCF, on the cell cycle status of blast progenitors was estimated by means of the titrated thymidine suicide test. The mechanism of Ara-C sensitivity modulation by SCF in relation to the cell cycle status of blast progenitors is discussed.

MATERIALS AND METHODS

Patients. Cells were obtained from peripheral blood of 15 patients with AML (M1–M7 except for M3 and M6 in the French-American-British classification) and five patients with ALL with informed consent. Profiles of the patients with AML are summarized in Table 1. Patients whose leukemic cells accounted for small proportions in the blood were in general not included in this study because a large number and high purity of blast cells were required for experiments. The patients with AML were all treated with an intensive individualized induction therapy using behenoyl cytosine arabinoside, daunorubicin (or instead, acracycinomycin in patient 15), 6-mercaptopurine, and prednisolone. This clinical protocol had been reported to produce high complete remission rates (35).

Cell Culture Procedures. T-cells were depleted from mononuclear cell fractions obtained from heparinized peripheral blood as described previously by using the two-cycle Ficoll-Hypaque procedure, the first followed by E-rosetting (17, 19–21, 22). These cells were immediately cultured or cryopreserved in liquid nitrogen. Fresh or thawed cells were cultured in suspension in αMEM (Gibco, New York, NY) containing 20% fetal calf serum (growth medium) at 37°C overnight to remove monocytes by plastic adherence. Blast populations thus obtained were washed in αMEM, resuspended in the growth medium, and used for experiments.

Leukemic blast colony formation was assessed using methylcellulose culture as described previously (primary clonogenic assay) (17, 19–21, 23). Blast cells were plated in each well of Linbro/Titertek microtitration 96-well plates (Flow Laboratories, McLean, VA) at a concentration of 104 cells per well in 0.1 ml of growth medium containing 0.8% methylcellulose and one of the four HGFs (SCF, IL-3, G-CSF, or GM-CSF); or two HGFs including SCF; or in the absence of added growth factors and then cultured in fully humidified air with 5% CO2 at 37°C. Colonies consisting of more than 20 cells were counted on days 5–7 of culture using triplicate cultures.

To assess self-renewal ability of blast progenitors, cells were cultured in suspension at a concentration of 105 cells/ml in 24-mm Linbro tissue culture multi-well plates (Flow Laboratories) in 1 ml of growth medium containing the same individual or combined HGFs as in the primary clonogenic assay or in the


absence of added growth factors. After 7 days, nonadherent cells were harvested, counted, and washed in the growth medium. To obtain plating efficiency after suspension culture, clonogenic assay was performed. The cells were plated in methylcellulose under the same culture conditions as for the primary clonogenic assay except that 10% of 5637-CM was used as a maximal growth stimulant in all cultures to detect a difference in effects of the HGFs used in suspension culture. Plating efficiency was obtained by counting colonies at days 5–7 of culture, and recovery of clonogenic cells after suspension culture, which is considered as a parameter of self-renewal of blast progenitors, was obtained by multiplying the plating efficiency by cell number after suspension culture.

**Morphology.** Cytospin specimen of the cells harvested from suspension culture were prepared and stained with May-Gr"undwald Giemsa for morphological observation.

**Cell Fractionation Procedures.** Blast cells obtained from four patients (nos. 1, 2, 3, and 7) were fractionated into subpopulations positive or negative for CD34, a stem cell–related antigen, by using an indirect immunomagnetic bead method. Blast cells were incubated with a monoclonal antibody against CD34, and after suspension culture in the growth medium for 24 h until the beads came off the CD34§ cells, and then plated in methylcellulose in the same way as for primary clonogenic assay at a concentration of 10^4 cells/well.

**Drugs Sensitivity Experiments.** Suspension culture was used to test the sensitivity to Ara-C of blast progenitors in AML because: (a) highly significant association has been found between clinical response and Ara-C sensitivity in suspension culture but not clonal assay, suggesting that the former is measuring a cell parameter important for treatment outcome (36); and (b) cytotoxicity of Ara-C is greater in suspension than in clonal assay (36). Tests for Ara-C sensitivity were performed as described previously (24). Blast cells were cultured in suspension in the absence or presence of HGFs, alone or in combination, with increasing concentrations of Ara-C or without drug. After 7 days, the cells were harvested; viable cells were counted with trypan blue staining, washed, and plated in methylcellulose with 10% of 5637-CM at 10^4 cells/plating. Efficiency of clonogenic cells was measured by counting colonies after another 5–7 days, and the number of surviving clonogenic cells/ml was calculated as a product of cell recovery multiplied by the plating efficiency. Clonogenic cell recoveries obtained in varying drug concentrations were recorded as the percentage of clonogenic cells in cultures without drug.

**Statistical Analysis.** Statistical analyses were all done using a microcomputer program for medical statistics (StatFlex; Nankodo, Tokyo, Japan). Group means were compared by Student’s t test for the probability of significant differences. Synergism was defined as growth stimulation by two cytokines in combination that significantly exceeds the sum of stimulation by the cytokines used separately, while other growth enhancement by the simultaneous use of two cytokines was considered an additive effect. An inhibitory interaction was defined as failure of the activity of two cytokines used in combination to reach that of either one or both of the cytokines used separately. Ara-C survival curves were computed by regression of clonogenic cell recovery against the dose of Ara-C. Drug sensitivity was then expressed for each curve as the D_{50} with SD from the fitted regression curves. Drug sensitivity was tested for probability of significant differences in the slopes of the survival curves for different growth factor conditions.

**Tritiated Thymidine ([3H]dThd) Suicide Test.** The proportion of blast progenitors in the S phase of the cell cycle after suspension culture in the presence of different HGFs was estimated by the [3H]dThd suicide test according to the methods described previously (37, 38) with modifications. Experiments were performed in two ways using cells from patients 3 and 7. Blast cells were cultured in 24-mm Linbro tissue culture multi-well plates (Flow Laboratories) in 1 ml of growth medium with G-CSF and SCF^2 alone or in combination at a concentration of 10^6 cells/ml for 24 h (experiment 1) or 6 days (experiment 2). In both experiments, the cells were then harvested, washed, resuspended in nucleoside-free aMEM (Gibco) containing 20% dextran-coated fetal calf serum, the same growth factor(s) used in the preceding suspension cultures, and 110 μg/ml [3H]dThd (specific activity, 25 Ci/mmol; Amersham International, Buckinghamshire, England) and recultured for 20 min or 24 h in experiments 1 and 2, respectively. The cells were then washed twice with growth medium and plated in 1 ml of growth medium containing 0.8% methylcellulose and 10% 5637-CM in 35-mm tissue culture dishes (Becton Dickinson) at a concentration of 2 × 10^5 cells/dish. Colonies were counted after 7 days using triplicate cultures. The survival of clonogenic cells was recorded as the percentage of clonogenic cells in the control cultures containing unlabeled thymidine (2.5 amol/ml) instead of [3H]dThd, a measurement of the proportion of blast progenitors that did not enter into the S phase of the cell cycle during exposure to [3H]dThd. These procedures were designed so that experiment 1 would reveal the immediate effects of HGFs on the cell cycle status of blast progenitors at certain time points, while experiment 2 would create a situation similar to that of the Ara-C experiments, where

### Table 1 Summary of patients and clinical data

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis (FAB)</th>
<th>WBC (10^3/mm^3)</th>
<th>Blasts (%)</th>
<th>Bone marrow blasts (%)</th>
<th>Response to therapy</th>
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<tr>
<td>1</td>
<td>38</td>
<td>M</td>
<td>M1</td>
<td>264,000</td>
<td>99</td>
<td>95</td>
<td>CR^1</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>M</td>
<td>MDS-M1</td>
<td>9,600</td>
<td>52</td>
<td>dry tap</td>
<td>PR</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>M</td>
<td>MDS-M1</td>
<td>111,000</td>
<td>94</td>
<td>94</td>
<td>CR</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>F</td>
<td>M1</td>
<td>12,900</td>
<td>78</td>
<td>94</td>
<td>CR</td>
</tr>
<tr>
<td>5</td>
<td>43</td>
<td>F</td>
<td>M2</td>
<td>39,100</td>
<td>73</td>
<td>61</td>
<td>CR</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>M</td>
<td>M2</td>
<td>17,400</td>
<td>84</td>
<td>83</td>
<td>CR</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>M</td>
<td>MDS-M2</td>
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<td>85</td>
<td>95</td>
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</tr>
<tr>
<td>8</td>
<td>78</td>
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<td>M4</td>
<td>13,600</td>
<td>63</td>
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<td>F</td>
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<td>95</td>
<td>96</td>
<td>CR</td>
</tr>
<tr>
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<td>46</td>
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<tr>
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<td>15</td>
<td>F</td>
<td>M7</td>
<td>14</td>
<td>50</td>
<td>50</td>
<td>CR</td>
</tr>
</tbody>
</table>

^1 Patients 1 and 9 in this table have appeared in Ref. 28 as patients 2 and 8, respectively, and patient 15 has appeared as case 1 in Ref. 20.

^2 FAB, French-American-British classification.

^3 CR, complete remission; PR, partial remission; NR, no response.

### Abbreviations

- SCF, stem cell factor
- HGF, hematopoietic growth factor
- AML, acute myelogenous leukemia
- IL, interleukin
- OM-CSF, granulocyte-macrophage colony-stimulating factor
- G-CSF, granulocyte colony-stimulating factor
- Ara-C, 1-β-D-arabinofuranosylcytosine
- Ara-CTP, 1-β-D-arabinofuranosylcytosine 5'-triphosphate
- αMEM, α-minimum essential medium
- [3H]dThd, tritiated thymidine
- D_{50}, dose required to reduce survival to 10% of the control
- MDS, myelodysplastic syndrome
- 5637-CM, medium conditioned by human bladder carcinoma cell line 5637.
blast cells were fully stimulated by incubation with growth factors for 7 days and killed by long exposure to a cell cycle-specific cytotoxic substance, thereby providing information on the proportion of all blast progenitors that entered into the cell cycle during the exposure period (the blast cells were exposed to [3H]dThd for 24 h because generation times of AML blast progenitors had been shown to be less than 24 h; Ref. 37).

**Cytokines.** Cytokines used are all recombinant forms provided by Genetics Institute (Cambridge, MA). The source of cytokines was medium conditioned by Chinese hamster ovary cells transfected with an expression plasmid containing complementary DNA encoding murine SCF truncated 5' to the transmembrane domain as described by Anderson et al. (3) or complementary DNA encoding human IL-3, GM-CSF, or G-CSF. Optimal concentrations were determined by titrating the cytokines in colony assay using blast cells from patients with AML as targets. The final concentrations of each cytokine used were: SCF, 2 units/ml (Mo-7 proliferation assay); IL-3, 200 ng/ml; GM-CSF, 100 ng/ml; and G-CSF, 300 units/ml (murine bone marrow cell colony assay).

**RESULTS**

**Effects of SCF in Methylcellulose Culture.** Leukemic blast colony formation was variably stimulated by SCF and the three other HGFs, IL-3, GM-CSF, and G-CSF, alone or in combination with SCF. We classified the patients into four groups according to the pattern of response of the blast progenitors to the individual cytokines (Fig. 1). This classification, although arbitrary, makes it easier to characterize the effects of SCF in comparison with other HGFs. In 11 of the 15 patients examined, SCF alone was active in stimulating colony formation (groups I-III). In 7 of these 11 patients, IL-3 was the most powerful stimulator, followed by GM-CSF, and then G-CSF or SCF (group I). Two of the other 4 patients were unique because their leukemic cells responded to each of the HGFs very similarly but very differently from the cells of all of the other patients (group II); G-CSF and SCF were the major stimulants of colony formation, whereas IL-3 and GM-CSF exhibited only modest activity in these patients. A third group of SCF-responders consisted of patients in which SCF was the most powerful stimulator of colony formation among the four HGFs (group III). The cells from the remaining four patients (group IV) did not show any significant response to SCF alone.

The outstanding effect of SCF was enhancement of colony formation, in terms of both number and size, when used in combination with other HGFs (Fig. 1). Synergistic or additive increases in the number of colonies were seen with at least one of the three combinations (SCF+IL-3, SCF+GM-CSF, and SCF+G-CSF) in most if not all patients (nos. 2, 3, 5-9, and 11-14; i.e., 11 of the 15 patients in total with synergism in 8 of these 11 patients). Particularly with the cells from patients 2, 3, 5, 7, and 8, synergism was evident with all three combinations of HGFs. In group III patients, however, no enhancement of colony formation by the use of combined HGFs was seen, and with the cells from patient 4, a decrease in colony numbers compared to the single HGFs, i.e., inhibitory interactions, were seen. In group IV patients, in which SCF alone had no significant effects, some combinations of SCF and another HGF resulted in either enhancement or inhibition of colony formation in all patients. Thus, SCF was active in a way in some way for all of the AML patients examined.

Blast cells from five patients with acute lymphoblastic leukemia were also tested for a response to SCF and other HGFs, alone or in combination, using the clonogenic assay in the same way as for AML patients, but no significant colony stimulation was observed with any of the HGFs.

**Effects of SCF in Suspension Culture.** In contrast to leukemic blast colony formation in methylcellulose, which is thought to reflect predominantly terminal divisions of blast progenitors, suspension culture provides favorable conditions for self-renewal (16, 36). Therefore, a comparison of clonogenic cell recovery after suspension culture with various HGFs shows the relative activity of each cytokine to support the self-renewal of blast progenitors. The data for nine patient samples are shown in Fig. 2. The response to the four HGFs was largely similar to the results of methylcellulose culture, i.e., IL-3 and, though not in all patients, GM-CSF were the major stimuliants for the cells from group I patients (nos. 1, 2, 5, 6, 9, 12, and 13) but were less active than SCF and G-CSF for cells from patients 3 and 7 (group II). SCF alone was active in stimulating growth in suspension of blast progenitors from most patients examined. When combined with other cytokines, enhanced growth of clonogenic cells was seen particularly with SCF plus G-CSF (patients 2, 3, 5, 6, 7, 9, and 13), but synergism was detected only in patients 5 and 9. The cells taken from the majority of the patients retained their blast morphology in suspension culture under all conditions, although the cells from some patients displayed incomplete granulation irrespective of the cytokines added, and no apparent differentiation toward mature blood cells was induced. The cell samples from five patients with acute lymphoblastic
leukemia showed no response to SCF or other HGFs, alone or even in combination, in suspension culture.

Response of Fractionated Blast Progenitors to SCF. To determine whether expression of CD34, an early stem cell-related antigen, is at all correlated with the response of blast progenitors to SCF, blast cells were separated into CD34+ and CD34- fractions using the immunomagnetic bead method, and the response of blast progenitors to HGFs was determined using clonogenic assay (Fig. 3). With rare exceptions, the patterns of response to the individual cytokines were not significantly different among the three populations, i.e., among unfractionated, CD34+, and CD34- cells from any of the patients. However, a difference in the overall magnitude of the response to these cytokines was detected between CD34+ and CD34- fractions in all patients. The response of CD34+ cells was higher than that of CD34- cells in three patient samples (nos. 1, 2, and 3), whereas the opposite result was obtained with cells from patient 7.

Modulation of Ara-C Sensitivity by HGFs. The observation that SCF is a potent growth enhancer in clonal culture led us to test the influence of SCF on the sensitivity of blast progenitors to Ara-C in vitro. Patient samples in which synergistic growth enhancement by HGFs was detected in culture were chosen; those without such interactions were used as controls. Then patient samples were tested for the sensitivity of blast progenitors to Ara-C. Typical survival curves of clonogenic cells are shown in Fig. 4 (patient 2) and Fig. 5 (patients 3 and 7). With cells from these three patients, synergism was seen with all three combinations, SCF plus IL-3 and GM-CSF or G-CSF in clonal culture, as described. With the cells from patient 2, these three combinations of HGFs all resulted in higher drug sensitivity than with each of the cytokines alone, whereas SCF alone displayed little effect in increasing drug sensitivity compared to that with no growth factor control and was less active than the other HGFs (Fig. 4). These data were correlated with the relative leukemic colony-stimulating activity.
of SCF and other HGFs. In this case, the cells taken at different disease stages, i.e., at the onset and the time of the first relapse, were used separately in experiments 1 and 2, respectively. The somewhat different sensitivity of blast progenitors to Ara-C in the presence of SCF in the two experiments may be ascribable to this fact, although growth characteristics in terms of the response to individual HGFs were not significantly different (data not shown).

Cells from patients 3 and 7 displayed unique responses to HGFs in culture as stated above. SCF and G-CSF were the major stimulants and combinations of these two yielded maximal stimulation of colony formation. The results of drug sensitivity experiments were consistent with this. Culture of the cells from patient 3 in the absence of added HGFs resulted in the lowest sensitivity to Ara-C, followed by IL-3 and GM-CSF (Fig. 5A). G-CSF and SCF were more effective than the former two, although the difference was small. Compared to the individual HGFs, all three combinations of HGFs including SCF resulted in much higher sensitivity to Ara-C. In the case of the cells from patient 7, SCF or G-CSF alone resulted in higher Ara-C sensitivity than did GM-CSF alone (Fig. 5B). The combination between SCF and GM-CSF resulted in higher sensitivity than did SCF or GM-CSF alone, and the combination of SCF and G-CSF had the maximal effect in sensitizing the blast progenitors. The results in patients 3 and 7 again largely paralleled the colony-stimulating activity of the HGF(s) in clonal culture.

The Ara-C sensitivity of blast progenitors from six patients studied, including three control patients in which cytokine combinations displayed no significant growth enhancement in either clonal or suspension cultures (patients 6 and 12) or only a slight additive effect in clonal culture (patient 9), are shown as D10 values in Table 2. As already described, the samples from patients 2, 3, and 7 displayed increased Ara-C sensitivity, i.e., decreased D10 values, when SCF was simultaneously used with another cytokine. There were statistically significant differences between the slopes of the survival curves of many cytokine combinations versus those of cytokines used alone. In contrast, in the other three patients, cytokine combinations resulted in D10 values little different from either one or both of the cytokines used alone.

Tritiated Thymidine Suicide Test. The cell cycle status of blast progenitors after suspension culture was assessed using samples from two patients (nos. 3 and 7) that exhibited significant sensitization to Ara-C with the combination of G-CSF and SCF. Colony formation was significantly inhibited by a 20-min exposure to [3H]dThd after
24-h culture under all growth factor conditions (Table 3). In both patients, the extent of inhibition was higher in the cultures with proportion of blast progenitors in the S phase of the cell cycle was of clonogenic cell survival than after a 20-min exposure in patient 3, under all growth factor conditions resulted in a much greater decrease when SCF and G-CSF were used together. Exposure of the blast cells to \(^{3}\mathrm{H}\text{dThd}\) for 24 h after 6 days of suspension culture with five of these patients displaying synergism with all three combinations of HGFs (SCF+IL-3, SCF+GM-CSF, and SCF+G-CSF).

DISCUSSION

Our present data demonstrate that SCF alone was substantially active in stimulating colony formation in clonal culture and in supporting self-renewal in suspension culture of AML blast progenitors in some, but not all, patients. Synergistic interactions with SCF and another HGF were also observed, particularly in clonal culture, with at least one of the HGF combinations in 8 out of the 15 patients studied, with five of these patients displaying synergism with all three combinations of HGFs (SCF+IL-3, SCF+GM-CSF, and SCF+G-CSF). The functional aspect of SCF as a synergistic factor in vitro is well known in normal hematopoiesis (3, 5–7, 10–13) as well as in leukemic stem cell growth (39–42). In contrast, samples without synergistic interactions displayed little such effect. These results suggest that CD34, a stem cell-related antigen, might distinguish subpopulations of cells that respond differently to HGFs, including SCF. Expression of CD34, however, yielded no particular correlation with growth characteristics, although the overall magnitude of the response to the HGFs of the two subpopulations was different. These results suggest that CD34 may be an antigen that is related in part to the blast progenitors in AML, either positively or negatively, but not to growth dependency on particular cytokines. Similar observations regarding the response of AML blast progenitors to HGFs, excluding SCF, have been made by other investigators (44). Further studies using other markers of leukemic cell subpopulations will be required to address these problems more precisely.

We (24) and other investigators (25–28) have demonstrated that the drug sensitivity of leukemic progenitors can be modified by HGFs in vitro. The present study demonstrated that combinations of HGFs including SCF that showed synergistic enhancement of colony formation resulted in higher Ara-C sensitivity, although some of the HGF combinations did not reach the level of statistical significance. In contrast, samples without synergistic interactions displayed little such effect. These results suggest that SCF, when combined with other HGFs, provides optimal growth stimulation and maximum sensitization to Ara-C in selected SCF-responsive patients and that SCF may be clinically useful for intensifying the cytotoxic effects of Ara-C. The finding that Ara-C sensitivity was intensified by HGF combinations, including SCF, in patients with AML originating in MDS is encouraging because MDS-related leukemias are usually refractory to therapy. Such patients might benefit from chemotherapy using SCF.

### Table 3: Survival of clonogenic cells after exposure to \(^{3}\text{H}\text{dThd}\)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Growth factor(s)</th>
<th>Incubation time with (^{3}\text{H}\text{dThd}) (h)</th>
<th>Colonies×10(^4) cells plated</th>
<th>(^{3}\text{H}\text{dThd}) (cpm)</th>
<th>(^{3}\text{H}\text{dThd}) (cpm)</th>
<th>Survival (%)</th>
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<tr>
<td>3</td>
<td>SCF</td>
<td>20 min</td>
<td>1725 ± 384</td>
<td>4000 ± 1005</td>
<td>43</td>
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<tr>
<td></td>
<td>G-CSF</td>
<td>20 min</td>
<td>2544 ± 312</td>
<td>4832 ± 443</td>
<td>32</td>
<td></td>
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<tr>
<td></td>
<td>G-CSF+SCF</td>
<td>20 min</td>
<td>815 ± 160</td>
<td>4834 ± 643</td>
<td>17</td>
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<tr>
<td></td>
<td>SCF</td>
<td>24 h</td>
<td>106 ± 24</td>
<td>5307 ± 1220</td>
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<tr>
<td></td>
<td>G-CSF</td>
<td>24 h</td>
<td>82 ± 16</td>
<td>5571 ± 261</td>
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<tr>
<td></td>
<td>G-CSF+SCF</td>
<td>24 h</td>
<td>53 ± 10</td>
<td>6440 ± 661</td>
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<tr>
<td>7</td>
<td>SCF</td>
<td>20 min</td>
<td>410 ± 88</td>
<td>1291 ± 342</td>
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<tr>
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<td>G-CSF</td>
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<td>381 ± 28</td>
<td>1145 ± 333</td>
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<td></td>
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<td>1648 ± 603</td>
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<tr>
<td></td>
<td>SCF</td>
<td>24 h</td>
<td>0</td>
<td>5770 ± 455</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G-CSF</td>
<td>24 h</td>
<td>0</td>
<td>3016 ± 272</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G-CSF+SCF</td>
<td>24 h</td>
<td>0</td>
<td>3800 ± 356</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Number of colonies represent mean ± SD of triplicate cultures.
Drug sensitivity modulation mechanisms that are not dependent on cell cycle changes have been demonstrated by other investigators. Retinoic acid and hydrocortisone were found by Lishner et al. (45) and Yang et al. (46), respectively, to influence Ara-C sensitivity without changing the cell cycle status of clonogenic cells. It has also been found that cells are regularly more Ara-C-sensitive when grown in the presence of G-CSF than in the presence of IL-3 or GM-CSF, irrespective of cell cycle status (38), and a function of G-CSF similar to that of G-CSF has been demonstrated using a leukemic cell line (47). Changes in the metabolic pathway of Ara-C evoked by HGFs are also thought to be involved in modification of Ara-C sensitivity. Ara-C is converted by deoxycytidine kinase to Ara-CTP, a biologically active metabolite, which inhibits DNA polymerase by competing with deoxyctydine triphosphate for incorporation into DNA (48). Bhalla et al. (28) have shown that GM-CSF and IL-3 in combination differentially raise intracellular Ara-C-TP levels and the Ara-C-TP: dCTP pool ratio in AML blasts versus normal bone marrow cells, resulting in selective cytotoxicity of Ara-C for leukemia cells. It is crucial to assess whether SCF has such differential effects on leukemic versus normal stem cells before it is used for patients with AML.

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REFERENCES


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Combinations of Stem Cell Factor with Other Hematopoietic Growth Factors Enhance Growth and Sensitivity to Cytosine Arabinoside of Blast Progenitors in Acute Myelogenous Leukemia

Yuka Inatomi, Keisuke Toyama, Steven C. Clark, et al.


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