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ORIGINAL ARTICLE

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Biology and clinical potential of stem-cell factor

Abstract Stem-cell factor (SCF) is a hematopoietic growth factor that acts on both primitive and mature progenitor cells. Preclinical studies have shown that recombinant SCF can protect against lethal irradiation, elicit multilineage hematopoietic responses and increases in bone marrow cellularity, and increase the number of circulating peripheral blood progenitor cells (PBPCs) in a dose-dependent manner. Both preclinical and early clinical studies using recombinant methionyl human SCF plus recombinant methionyl human granulocyte colony-stimulating factor (Filgrastim) have demonstrated increased PBPC mobilization as compared with the use of either factor alone. These data suggest a clinical role for the combination.

Key words Stem-cell factor · Peripheral blood progenitor cells · Mobilization · High-dose chemotherapy · CD34 cells

Introduction

The growth and maturation of hematopoietic cells in the bone marrow are controlled by the action of hematopoietic growth factors, also called colony-stimulating factors (CSFs) [19, 20] (Fig. 1). More than 20 hematopoietic growth factors have been identified, and some have been cloned and produced using recombinant DNA techniques. The factors appear to have different roles and can affect the proliferation, differentiation, and maturation of progenitor cells as well as the activation and survival of mature cells.

Stem-cell factor (SCF), variously called c-kit ligand, Steel factor, and mast-cell growth factor, is the natural ligand for the c-kit tyrosine kinase receptor and has been found on various cell types, including melanocytes, neurons, germ cells, mast cells, and hematopoietic cells. SCF acts on both lineage-committed and nonlineage-committed hematopoietic progenitor cells, mature hematopoietic progeny such as natural killer cells, and mast cells [12, 15, 16, 29].

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Biochemical characteristics of SCF

The soluble form of SCF is a glycosylated protein with a molecular weight of approximately 45 kDa in its dimeric form, including the carbohydrate moieties [31]. The protein sequence is highly conserved across species, and there is >80% amino acid homology between the murine and human forms. SCF is found in two forms in great abundance in the stroma of bone marrow: a membrane-bound form and a soluble form [28]. Human SCF is encoded by a gene on chromosome 12, and the full-length, membrane-bound form is known to have a 25-amino-acid leader sequence, an extracellular domain of 185 amino acids, a transmembrane segment of 27 amino acids, and a 36-amino-acid cytoplasmic domain. The most prevalent endogenous soluble form of SCF appears to be 165 amino acids in length and is processed from the full-length, membrane-bound form by proteolytic cleavage. A second membrane-bound form exists that lacks the proteolytic cleavage site.

The SCF receptor, c-kit, is a class III tyrosine kinase protein receptor. When the SCF ligand binds to the c-kit

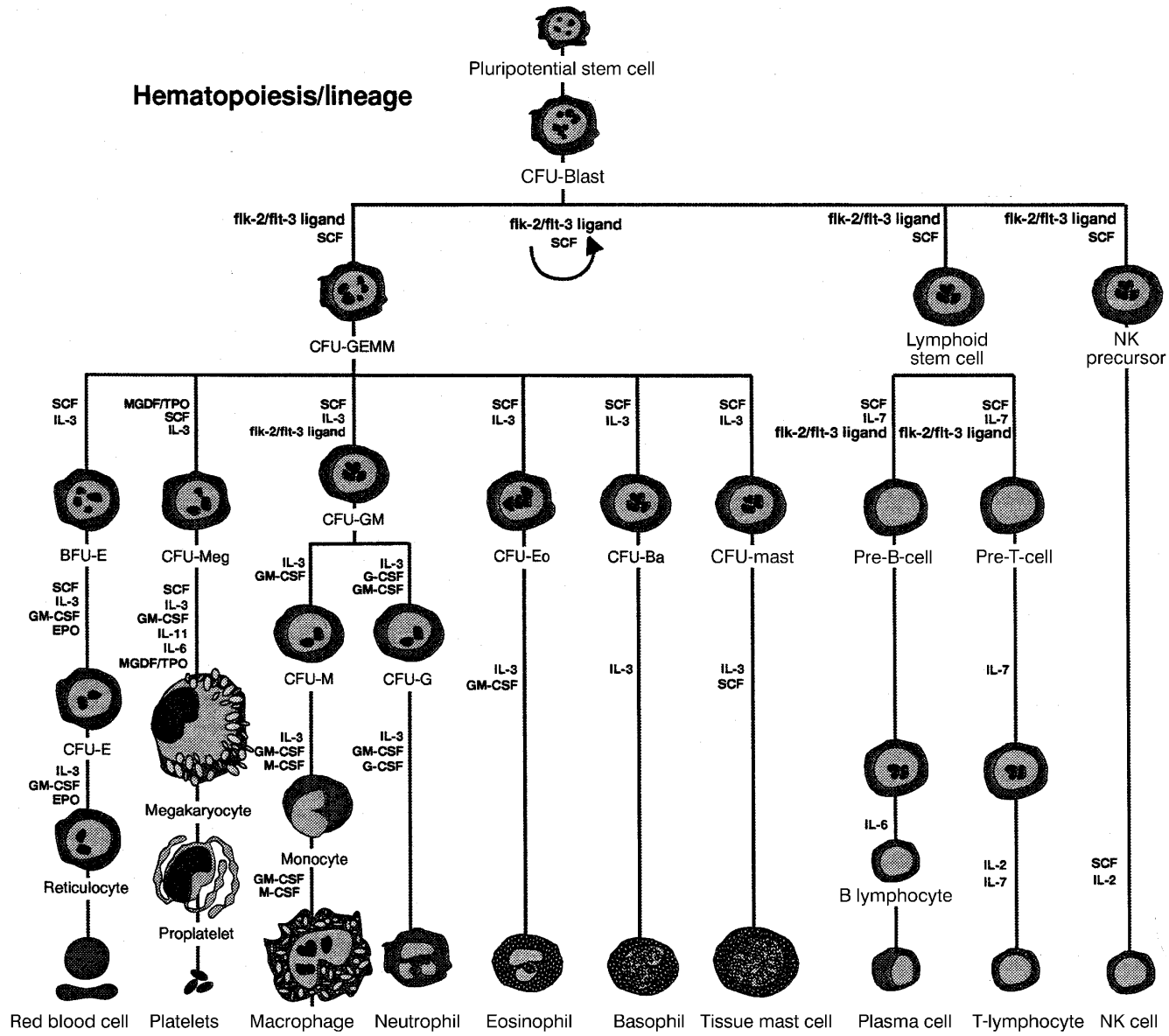

Fig. 1 Schematic hematopoietic tree showing the control of hematopoiesis as presently known. There appears to be a hierarchy of development, with SCF, flk-2/flt3 ligand, and IL-3 influencing early multipotential progenitor cells. Other factors, such as EPO, G-CSF, GM-CSF, MGDF/thrombopoietin (TPO), and macrophage colony-stimulating factor (M-CSF), appear to act on later progenitors. SCF is thought to act on a subset of multipotent stem cells, enhancing their proliferative response to other hematopoietic growth factors

receptor, the tyrosine kinase is activated, causing autophosphorylation of tyrosine residues and conformational changes [33].

SCF was purified in 1989 and the human gene was first cloned at Amgen Inc. (Thousand Oaks, Calif., USA) [31, 32]. Recombinant methionyl human SCF (r-metHuSCF) is a noncovalent, dimeric protein produced in Escherichia coli. It has one extra amino acid, the N-terminal methionine, as compared with the soluble endogenous form and is nonglycosylated.

Biological effects of SCF

SCF has a major role in hematopoiesis because it stimulates primitive, undifferentiated blast cells and works in synergy with other hematopoietic growth factors [5, 17]. In vitro studies have shown that r-metHuSCF used alone has limited colony-stimulating activity. However, colony formation is greatly increased when r-metHuSCF is used with other hematopoietic growth factors [5, 13, 14, 17]; in all combinations, both the number of colonies and the number of cells per colony were increased. The combination of rHuSCF with rHu interleukin (IL)-3, rHu granulocyte colony-stimulating factor (G-CSF), rHu granulocyte-macrophage colony-stimulating factor (GM-CSF), or rHu erythropoietin (EPO) increased the frequency of colony

formation as compared with that induced by each of the growth factors alone [18].

In a murine model, recombinant rodent (rr) SCF pretreatment protected against lethal irradiation [33], and in a canine model, recombinant canine (rc) SCF enhanced survival and accelerated hematologic recovery from total body irradiation [31]. When r-metHuSCF at 200 μg/kg per day was given to normal baboons for ≤28 days, it elicited a multilineage response in peripheral counts and increased bone marrow cellularity [1, 2]. In the same model, r-metHuSCF given at 25–200 μg/kg per day for ≤28 days increased circulating peripheral blood progenitor cell (PBPC) counts as much as 300-fold in a dose-dependent manner. These PBPCs were capable of rescuing baboons from otherwise lethal irradiation.

SCF is capable of exerting synergistic activity with endogenous or exogenous cytokines in vivo as demonstrated in preclinical studies. When recombinant forms of SCF are used in combination with recombinant forms of G-CSF, PBPC mobilization is increased as compared with the mobilization induced by either cytokine alone. This has been demonstrated in mice, dogs, and baboons and is discussed in more detail below.

Mobilization of PBPCs

As successful as autologous bone marrow transplantation (BMT) is in restoring hematopoietic function after myeloablative chemotherapy, it is limited by the rate of hematopoietic recovery achieved, the requirement for general anesthesia for harvest of hematopoietic cells, and its labor intensiveness and cost. Some of these limitations can be circumvented by PBPC transplantation. This technique has been used in place of BMT or to supplement BMT, and it can potentially extend the benefits of transplantation to patients unwilling to undergo standard bone-marrow harvest procedures and to patients who have inadequate bone marrow for harvesting due to previous pelvic irradiation, bone marrow hypocellularity, or tumor infiltration into the bone marrow. In contrast to BMT, PBPCs are easily collected without anesthesia. Most importantly, engraftment with PBPCs is generally more rapid than engraftment after BMT.

There are several potential ways to mobilize PBPCs for harvesting for transplantion, including use of chemotherapy alone, use of CSFs alone, use of chemotherapy and CSFs, combinations of CSFs, and sequential use of CSFs. Several theories have been proposed to explain the possible mechanism for progenitor cell release, including down-regulation of, or a functional change in, adhesion molecules; proliferation of progenitor cells; and delay in the clearance of progenitor cells from the blood.

Cyclophosphamide has been shown to be effective in increasing the number of circulating stem cells in patients with myeloma, lymphoma, and solid tumors [26] and is one of the chemotherapeutic agents that has been most extensively studied for its use in PBPC mobilization. However, use of cyclophosphamide is not without risk, and death has been reported as a complication of this technique when cyclophosphamide doses of 4–7 g/m^2 have been used without r-metHuG-CSF (Filgrastim) support [25, 27].

Much work has been done using rG-CSF to mobilize PBPCs, and it is effective for this purpose. However, the combination of r-metHuSCF with rG-CSF results in an increase in the number of colony-forming cells detected as compared with the use of rG-CSF alone [6].

Studies using rrSCF alone at 25 μg/kg have shown that this regimen is not effective for mobilization. Therefore, studies of the combination of rrSCF and rHuG-CSF were done to determine the ability of these factors to mobilize PBPCs with bone-marrow-repopulating ability in mice [6, 18, 30]. Synergistic increases in circulating low-density mononuclear cells, granulocyte-macrophage colony-forming cells (GM-CFCs), and high-proliferative-potential colony-forming cells (HPP-CFCs) were observed when the rrSCF and rG-CSF combination was used. Mice treated with the rrSCF and rG-CSF combination showed an approximately 1.5-fold increase in the number of circulating white blood cells, a 5-fold increase in GM-CFCs, and a 2-fold increase in HPP-CFCs as compared with those treated with rG-CSF alone [6].

The combination of rcSCF and rcG-CSF dramatically increased the numbers of PBPCs in a canine model [21, 22]. Peripheral blood mononuclear cells mobilized by the cytokines alone or in combination were capable of rescuing lethally irradiated dogs, whereas an equal number of peripheral blood mononuclear cells from control animals (no cytokine pretreatment) were not. Additionally, the time to engraftment, as defined by the presence of 0.5×10^9 granulocytes/l in the peripheral blood after transplant, was shown to be reduced when the combination was compared with either rcG-CSF alone or high-dose rcSCF alone. There was a trend toward more rapid platelet engraftment to 20×10^9/l in animals receiving the cytokine combination, but this was not statistically significant.

In baboons, low doses of r-metHuSCF caused no change in the number of peripheral white blood cells when given alone but were found to increase significantly the numbers of peripheral white blood cells and multilineage hematopoietic progenitor cells when given in combination with r-metHuG-CSF (Filgrastim) [3]. The increased progenitor-cell population included megakaryocyte progenitor cells (MK-CFC), suggesting that the use of r-metHuSCF/r-metHuG-CSF-mobilized PBPC may produce improved engraftment of platelets after transplantation.

Early clinical results

Phase I clinical studies using r-metHuSCF at doses of 5, 10, 25, and 50 μg/kg per day in patients with advanced non-small-cell lung cancer [8] and breast cancer [9] have been performed. Recombinant r-metHuSCF has shown synergy with r-metHuG-CSF in clinical studies of PBPC mobilization and transplantation. It was reported [8, 9] that at higher

doses, several patients developed allergic-like reactions, including generalized urticaria and/or throat tightness, and 50 μg/kg per day was thought to represent the maximum tolerated dose without premedication. Other common side effects often seen with cytokines, such as fever, were not observed. However, the use of r-metHuSCF in phase II studies was restricted to doses of <50 μg/kg per day.

The mean frequency of bone-marrow $CD34^+$ cells increased, as did the mean proportion of cells with proliferation-associated nuclear protein ($P<0.003$ and $P \leq 0.01$, respectively). Patients with high-risk or advanced breast cancer were enrolled into a study of r-metHuSCF-mobilized + r-metHuG-CSF-mobilized PBPCs for transplantation [10]. All patients had received previous chemotherapy. Patients were given r-metHuSCF alone, r-metHuG-CSF alone, or the combination of r-metHuSCF plus r-metHuG-CSF. Several r-metHuSCF doses (5–30 μg/kg per day) and schedules of administration (7, 10, and 13 days) were tested.

Leukapheresis was performed on 3 consecutive days and the harvested PBPCs were infused after high-dose chemotherapy with a myeloablative regimen of cyclophosphamide, cisplatin, and carmustine. Supportive therapy with r-metHuG-CSF at 10 μg/kg per day was started on the day of PBPC transplantation and continued until the patient's neutrophil count had reached $\geq 5 \times 10^9$/l for 3 consecutive days or $\geq 10 \times 10^9$/l for 1 day. The combination of r-metHuSCF and r-metHuG-CSF mobilized a clinically useful number of progenitor cells, as measured by $CD34^+$ cell, GM-CFC, and erythroid burst-forming unit counts. In these patients, all of whom were screened for allergy history and received antihistamine premedication, generalized urticaria and other allergic-like reactions were rare. In general, r-metHuSCF was well tolerated.

Importance of CD34 cells

The CD34 antigen was first described by Civin et al. [7] and is expressed on all hematopoietic progenitor cells, including the pluripotent hematopoietic stem cells. These cells represent approximately 0.5–2% of the mononuclear cells in the bone marrow and $<0.1\%$ of the mononuclear cells in the peripheral blood unless they have been mobilized [4, 11, 24].

Clinical studies have shown that $CD34^+$ cells, which can be positively selected from blood, bone marrow, and umbilical cord blood, are the cells capable of long-term engraftment [23]. In addition, the number of $CD34^+$ cells infused after high-dose chemotherapy appears to be a useful predictor of the time to engraftment, especially for platelet recovery. Reported target levels of $CD34^+$ cells for transplantation range from 2×10^6 to 8×10^6/kg patient weight.

Conclusions

Its biological properties suggest that r-metHuSCF may have clinical utility in settings in which there is synergistic activity with other hematopoietic growth factors. Recombinant-metHuSCF is anticipated to be useful for mobilizing progenitor cells for transplantation after high-dose chemotherapy, ex vivo gene transfection, ex vivo expansion and differentiation of hematopoietic progenitor cells, and enhancement of hematopoiesis in various bone-marrow failure states. In PBPC transplantation, the combination of r-metHuSCF and r-metHuG-CSF appears to mobilize more $CD34^+$ cells than r-metHuG-CSF alone and may lead to a reduction in apheresis requirements. In addition to potentially reducing the number of apheresis procedures, the increase in PBPC yields achieved with r-metHuSCF plus r-metHuG-CSF may be useful in multicycle PBPC transplants, extension of transplantation techniques in heavily pretreated patients, improvement in hematopoietic recovery of heavily pretreated patients, and ex vivo manipulations involving cell loss (i.e., CD34 selection, tumor purging, ex vivo expansion, and gene transduction).

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