Distribution of Human Granulocyte Colony-stimulating Factor Receptors on Hematopoietic and Nonhematopoietic Tumor Cell Lines

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Introduction of two tyrosine residues into human granulocyte colony-stimulating factor (G-CSF) allowed us to obtain radioiodinated material efficiently. By using this material, specific receptors for human G-CSF were analyzed. It was found that human G-CSF receptors were expressed on several myelocytic and monocytic leukemia cell lines. Furthermore, they were also expressed on two choriocarcinoma cell lines. However, the human G-CSF receptors were not detected on other nonhematopoietic tumor cell lines examined, indicating that expression of G-CSF receptors is strictly limited as compared with receptors for granulocyte/macrophage colony-stimulating factor (GM-CSF), which are widely distributed on nonhematopoietic tumors.

Key words: Granulocyte colony-stimulating factor receptor — Site-directed mutagenesis — Scatchard analysis

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein which stimulates the growth of hematopoietic progenitors¹ and enhances the function of mature neutrophils² *in vitro*. Systemic administration of G-CSF was reported to stimulate markedly the production of granulocytes *in vivo*.^{3,4} The biological actions of G-CSF are mediated by binding to specific receptors on the surface of myeloid precursors and granulocytes. Murine G-CSF receptors with specific affinity have been identified by the use of radioiodinated murine G-CSF.⁵⁻⁷ However, little work has been done on human G-CSF receptors using human radioiodinated G-CSF except for reports with endothelial cells,⁸ placenta,⁹ cells from patients with hematopoietic disease,^{10,11} normal neutrophils,^{11,12} and hematopoietic tumor cell lines.¹⁰ Because of the current clinical trials of human G-CSF for increasing the level of granulocytes in patients with granulocytopenia after cancer chemotherapy, it is important to identify the distribution of the G-CSF receptors on hematopoietic tumor cells as well as nonhematopoietic tumor cells. Therefore, we looked for the specific receptors for human G-CSF on human hematopoietic and nonhematopoietic cell lines, and found that specific receptors for G-CSF were expressed on several hematopoietic cell lines and two choriocarcinoma cell lines.

In order to obtain radioiodinated human G-CSF, mutated G-CSF, which was named YPY-G-CSF, was prepared by site-directed mutagenesis. In the YPY-G-CSF, the first and third amino acids, threonine and leucine residues, respectively, were both substituted with

tyrosine. It had the same biological activity as intact G-CSF. YPY-G-CSF purified to homogeneity was radioiodinated to high specific activity using the lactoperoxidase method. Four μ l of 500 μ g/ml YPY-G-CSF and 2 μ l of 20-fold-diluted Enzymobeads (Nippon Bio-Rad Laboratory, Tokyo) were added to 10 μ l of 0.2 M sodium acetate buffer (pH 6.0) and the mixture was incubated with 18.5 MBq (0.5 mCi) of Na¹²⁵I for 5 h at 4°C. After the incubation, the reaction was quenched by adding 30 μ l of 100 μ g/ml Na₂S₂O₅ and 30 μ l of 0.1% NaI. Radioiodinated YPY-G-CSF was separated from the reactants by passage through a PD-10 column (Pharmacia, Uppsala) with 10 mM sodium acetate buffer (pH 4.0) containing 0.1% bovine serum albumin (BSA) and 0.004% Tween 20. The ¹²⁵I-YPY-G-CSF migrated as a single species with an apparent molecular weight of 18 kDa on analysis by autoradiography after SDS-PAGE. The efficiency of radioiodination of G-CSF was greatly enhanced by the presence of two tyrosine residues. The specific radioactivities of ¹²⁵I-YPY-G-CSF were determined by self-displacement analysis¹³ using U937 cells, and ranged from 2.5×10^5 to 5.0×10^5 cpm/ng for different preparations.

All cell lines were grown in RPMI1640 medium containing 10 to 20% fetal bovine serum. All hematopoietic cell lines were supplied by the Japanese Cancer Research Resources Bank. Nonhematopoietic cell lines tested were as follows; Lu24, Lu65, Lu130, Lu134, Lu139, Lu140, Lu141, Lu143, PC7, PC9, PC10, PC14 and A549 were derived from lung cancer (kindly provided by Dr. J.

Yokota); MCF7, BT20 and ZR75-1 were from breast cancer (kindly provided by Dr. K. Yamaguchi); KATO-III, MKN1, MKN28, MKN45 and MKN74 were from stomach cancer (except for KATO-III, kindly provided by T. Motoyama); Caski was from uterus cervical cancer; OST-1 was from osteosarcoma (kindly provided by Dr. S. Higaki); HuH7 (supplied by the Japanese Cancer Research Resources Bank) was from hepatoma; LS180 and HTB38 were from colon cancer; BeWo and JEG3 were from choriocarcinoma. Hematopoietic and nonadherent nonhematopoietic tumor cells were collected and washed twice with binding buffer (DMEM containing 0.1% BSA, 0.02% sodium azide and 25 mM Hepes, pH 7.5). The cells were incubated with 125 I-YPY-G-CSF after resuspending them in 100 μ l of the binding buffer with or without an excess of unlabeled G-CSF. After incubation for 3 h at 15°C, duplicate 40- μ l cell suspensions were transferred onto 100 μ l of fetal bovine serum in 0.4-ml polyethylene centrifuge tubes. Adherent nonhematopoietic tumor cells were plated in 12-well plates with 1 ml of RPMI1640 containing 10% fetal bovine serum. The cells were washed twice with binding buffer and incubated with 125 I-YPY-G-CSF in 300 μ l of binding buffer for 3 h at 15°C. After incubation, the cells

were washed three times with the binding buffer and solubilized with 500 μ l of phosphate-buffered saline (PBS). Both solubilized cell lysate and PBS used for washing were transferred to counting tubes.

The specific bindings of 125 I-YPY-G-CSF to U937 cells were inhibited by intact G-CSF and YPY-G-CSF but not by other hematopoietic factors including GM-CSF, interleukin-3 and erythropoietin. The inhibitions of 125 I-YPY-G-CSF binding to U937 cells by intact G-CSF and YPY-G-CSF were essentially the same as regards concentration dependence (Fig. 1). Table I shows the results of analysis for G-CSF receptors on human hematopoietic cell lines. Within the group of cells of myeloid and monocytoid lineage, G-CSF receptors were found on U937, THP-1, KY821 and KG-1 cells. Scatchard analyses of all these four cell lines revealed the existence of a single class of binding sites (data not shown). No receptors for G-CSF were detected on cells of lymphoid lineage. Table II shows the results of analysis for G-CSF receptors on human nonhematopoietic cell lines. Among

Fig. 1. Inhibition of specific 125 I-YPY-G-CSF binding to U937 cells by unlabeled YPY-G-CSF and intact G-CSF. Binding experiments were performed as described in "Materials and Methods." Nonspecific binding was subtracted from total binding to determine specific binding, and was approximately 30% of the total binding. The specific 125 I-YPY-G-CSF binding was expressed as a percentage of the specific binding measured in the absence of any added growth factors. Intact G-CSF (○), YPY-G-CSF (●)

Table I. Distribution of G-CSF Receptors on Human Hematopoietic Tumor Cell Lines

Designation	Characteristics	Sites/cell	K_d (pM)
HL-60	promyelocytic leukemia	< 20	—
KG-1	myelogenous leukemia	660	200
KU812	myelogenous leukemia	< 20	—
THP-1	monocytic leukemia	1200	260
U937	monocytic tumor	1800	330
KY821	leukemia	990	110
HEL	erythroleukemia	< 20	—
K562	erythroleukemia	< 20	—
BALL-1	B cell leukemia	< 20	—
MT-1	T cell leukemia	< 20	—
MOLT-4F	T cell leukemia	< 20	—

Receptor numbers and K_d values were calculated from binding experiments and subsequent Scatchard analysis.

Table II. Distribution of G-CSF Receptors on Human Non-hematopoietic Tumor Cell Lines

Tumor	Number of cell lines expressing G-CSF-R	Number of tested cell lines
Lung	0	13
Breast	0	3
Stomach	0	5
Uterus	0	1
Osteosarcoma	0	1
Hepatoma	0	1
Colon	0	2
Choriocarcinoma	2	2

28 cell lines investigated, G-CSF receptors could only be detected on two choriocarcinoma cell lines, BeWo and JEG3. Scatchard analyses of both cell lines revealed the existence of a single class of binding sites. For BeWo cells, the calculated K_d was 380 pM with 3,800 specific binding sites/cell, and for JEG3 cells the calculated K_d was 470 pM with 4,800 specific binding sites/cell.

Radioiodination of human G-CSF has been tried by several groups with little success, and thus only a few reports using mutant G-CSF have been published.^{9, 10, 12)} We have isolated a YPY-G-CSF with similar biological activity to that of the native molecule, and this material allowed us to obtain radioiodinated G-CSF in a reproducible manner. In this report, we have demonstrated the presence of specific receptors for G-CSF on several myeloid and monocytoid cell lines. Recently, Park *et al.* reported the distribution of G-CSF receptors on human hematopoietic cell lines, showing that receptors for G-CSF were present on U937 cells.¹⁰⁾ We detected specific receptors for G-CSF on THP-1, KY821 and KG-1 cells as well as U937 cells. In Park *et al.*'s report, THP-1 and KY812 cells were not investigated, and no receptors for G-CSF were found on KG-1 cells. Furthermore, G-CSF receptors were not detected on HL-60 cells, although the cell line was reported to respond to G-CSF.¹⁴⁾ It is possible that the cell lines used in their studies were different strains from those used in this study. We have also demonstrated the expression of G-CSF receptors on

nonhematopoietic choriocarcinoma cell lines, BeWo and JEG3. The presence of G-CSF receptors was detected on two of two tested choriocarcinoma cell lines, although no receptors for G-CSF could be detected on other non-hematopoietic cell lines. It was also reported that G-CSF receptors were expressed on human placental membrane.⁹⁾ Concerning the biological effects on hematopoietic progenitors and mature neutrophils, there are some similarities between G-CSF and GM-CSF, but the GM-CSF receptors were observed on 25% of examined nonhematopoietic tumor cell lines¹⁵⁾ and, moreover, on three of three choriocarcinoma cell lines¹⁶⁾ and human placental membrane.¹⁷⁾ These findings suggest that G-CSF receptors may be generally expressed on chorionic cells, like GM-CSF receptors, and that the distribution of human G-CSF receptors on non-hematopoietic cell lines is more restricted than that of human GM-CSF receptors. These results indicate that G-CSF is a lineage-specific growth factor and may be widely applicable for increasing the level of granulocytes in patients treated with cancer chemotherapy, with rare exceptions.

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