Stem cell factor induces outgrowth of c-kit-positive neurites and supports the survival of c-kit-positive neurons in dorsal root ganglia of mouse embryos

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SUMMARY

The c-kit receptor tyrosine kinase is highly expressed by about 10% of the neurons in the dorsal root ganglia (DRGs) of mouse embryos. We investigated the in vitro effect of stem cell factor (SCF), the ligand for c-kit receptor, on DRGs. Recombinant murine SCF (rmSCF) induced the outgrowth of c-kit-positive neurites from DRGs of normal (+/+) embryos. The effect of SCF was dose dependent and completely abolished by anti-c-kit ACK2 monoclonal antibody (mAb). Some neurites whose outgrowth was induced by nerve growth factor (NGF) were c-kit-positive, but anti-NGF mAb did not inhibit the rmSCF-induced neurite outgrowth. rmSCF did not induce neurite outgrowth from DRGs of W/W embryos that did not express c-kit receptors on the cell surface and of W42/W42 mutant embryos that expressed c-kit receptors without tyrosine kinase activity. rmSCF also had a trophic effect on c-kit-positive neurons in the culture of dissociated DRG cells. Most c-kit-positive neurons appeared to respond to NGF as well, and the SCF-responsive subpopulation represented about 10% of NGF-responsive neurons. rmSCF did not support the survival of DRG neurons from embryos of W/W and W42/W42 genotypes. These results suggest that the stimulus through the c-kit receptor tyrosine kinase has an important role in development of the peripheral nervous system.

Key words: neurite outgrowth, neurotrophic factor, stem cell factor, c-kit receptor, dorsal root ganglion, Sl locus, W locus, nerve growth factor

INTRODUCTION

The c-kit receptor tyrosine kinase is encoded by the W locus in mice (Chabot et al., 1988; Geissler et al., 1988). The ligand for c-kit receptor has been designated as stem cell factor (SCF), steel factor or mast cell growth factor, and is encoded by the Sl locus in mice (Williams et al., 1990; Flanagan and Leader, 1990; Zesbo et al., 1990; Huang et al., 1990). Hereafter we refer to the ligand as SCF. It has the transmembrane domain and appears to be a cell surface protein. However, recombinant SCF lacking the transmembrane domain has apparent functions both in vivo and in vitro (Tsai et al., 1991; Godin et al., 1991; Dolci et al., 1991; Matsui et al., 1991). Analyses of W and Sl mutant mice have shown that the interaction between the c-kit receptor and SCF is indispensable for proper development of melanocytes (Mayer and Green, 1968; Silvers, 1979), erythrocytes (Russell and Bernstein, 1968; Russell, 1979), mast cells (Kitamura et al., 1978; Kitamura and Go, 1979) and germ cells (Nakayama et al., 1988; Kuroda et al., 1988). These cells or their precursors express c-kit receptor on the surface, whereas cells surrounding them express SCF and appear to support their migration, proliferation or differentiation.

Recent in situ hybridization studies revealed that the c-kit receptor and SCF are highly expressed by some neurons in mice and rats (Matsui et al., 1990; Orr-Urtreger et al., 1990; Keshet et al., 1991; Motro et al., 1991; Morii et al., 1991; Hirota et al., 1992). Their expression is highly localized and there are synaptic connections between c-kit-positive and SCF-positive neurons (Hirota et al., 1992). These findings suggest that the c-kit receptor and SCF are involved in the development of neuronal connections. In this study, we directly demonstrated the function of the c-kit receptor and SCF in neural development. Since previous studies have shown that dorsal root ganglia (DRGs) of mice express c-kit mRNA (Orr-Urtreger et al., 1990; Motro et al., 1991; Keshet et al., 1991), we cultured DRGs from normal (+/+) and mutant (W/W, W42/W42, Sl/Sl) mouse embryos and examined the effect of recombinant murine SCF (rmSCF) on neurite outgrowth from these DRGs. We also examined the trophic effect of rmSCF on dissociated DRG neurons from +/- and mutant (W/W, W42/W42, Sl/Sl) mouse embryos.
**MATERIALS AND METHODS**

**Mouse embryos**

Mice of $\text{WB-}(+/+, \text{W}+/+, \text{Sl}+/+)$ and $\text{C57BL/6-}(+/+, \text{W}^{\text{Sl}}+/+, \text{Sl}^{\text{W}}+/+)$ were maintained in our laboratory. The $\text{W}$ mutant allele results in a deletion of the transmembrane domain of the $c$-kit receptor. Mast cells of $\text{W/W}$ genotype completely lack $c$-kit receptors on the surface (Nocka et al., 1990; Reith et al., 1990). The $\text{W}^{2}$ mutant allele is a point mutation at the tyrosine kinase domain of the $c$-kit receptor, which abolishes the tyrosine kinase activity (Tan et al., 1990). The $\text{Sl}$ mutant allele is a large deletion of the $\text{Sl}$ locus. Fibroblasts of the $\text{Sl}/\text{Sl}$ genotype do not produce SCF at all (Huang et al., 1990; Zesbo et al., 1990). The $\text{Sl}^{2}$ mutant allele results in a deletion of transmembrane and intracellular domains of SCF (Flanagan et al., 1991).

Embryos of $+/+$ genotype were obtained by mating either $\text{WB-}+/+$ or $\text{C57BL/6-}+/+$ parents. Embryos of various mutant genotypes were obtained by mating the corresponding heterozygous parents. The time of gestation was calculated by considering the morning after mating as 0.5 day post coitum (p.c.). The mothers were anesthetized by ether and killed by exsanguination 15.5 or 18.5 day p.c. and the embryos were dissected out. Homozygous mutant embryos at 18.5 day p.c. were determined by the apparent decrease of mast cells in the skin (Hayashi et al., 1985). Genotypes of $\text{W/W}$, $\text{Sl}/\text{Sl}$ and $\text{Sl}^{2}/\text{Sl}^{2}$ embryos at 15.5 day p.c. were determined by grafting skin pieces of embryos under the kidney capsule of congenic $+/+$ recipients according to Niwa et al. (1991). Development of white hair alone was considered to indicate $\text{W/W}$, $\text{Sl}/\text{Sl}$ or $\text{Sl}^{2}/\text{Sl}^{2}$ genotype. Since the coat color of heterozygous $\text{W}^{2}/\text{+/+}$ mice is almost white (Tan et al., 1990), determination of the $\text{W}^{2}/\text{+/+}$ genotype from hair color of grafted skin pieces was difficult. Therefore, the $\text{W}^{2}/\text{+/+}$ genotype of mouse embryos at 15.5 day p.c. was determined by sequencing the $c$-kit gene fragment (nucleotide 1707 through 2678) which was reverse transcribed and amplified by polymerase chain reaction (PCR) as described by Tono et al. (1992).

**Chemicals, factors and antibodies**

Serum-free medium (Cosmedium) purchased from Cosmo Bio (Tokyo, Japan) was supplemented with human transferrin (100 $\mu$g/ml, Sigma Chemical Co., St Louis, MO), bovine insulin (5 $\mu$g/ml, Sigma) and sodium selenite (5 ng/ml, Sigma) and used throughout the present study. The rmSCF was a generous gift of Kirin Brewery Company Ltd (Tokyo, Japan). Nerve growth factor (NGF) 2.5 S purified from mouse submaxillary glands was purchased from Boehringer Mannheim (Mannheim, Germany). Anti-$c$-kit ACK2 monoclonal antibody (mAb) that recognizes the extracellular domain of murine $c$-kit receptor was a generous gift from Dr. S. I., Nishikawa of Kumamoto University Medical School (Kumamoto, Japan). Anti-murine NGF mAb was purchased from Boehringer Mannheim.

**Histological specimens**

Embryos were frozen in liquid nitrogen. Sagittal sections of 6 $\mu$m in thickness were prepared and fixed in acetone for 10 minutes. The specimens were incubated with ACK2 mAb (20 ng/ml) for 42 hours at 4°C, then with biotinylated goat anti-rat IgG antibody (1:300, DAKO-PATTS, Copenhagen, Denmark), and finally with streptavidin-biotin-peroxidase complex (1:200, Vector Laboratories, Burlingame, CA). The binding of the streptavidin-biotin-peroxidase complex was visualized with diaminobenzidine. The specimens were counterstained with hematoxylin. Neurons were virtually indistinguishable from non-neuronal cells in frozen sections. The numbers of total cells and those stained with ACK2 mAb were counted using a square micrometer attached to an eyepiece.

**Assay of neurite outgrowth**

DRGs were dissected from all segments of mouse embryos harvested aseptically at 15.5 day p.c. Thereafter, 15 to 20 DRGs in Hank’s solution were placed on 35 mm plastic dishes (Corning, Tokyo, Japan) coated with poly-L-lysine (Sigma). The DRGs were cultured in the above-mentioned serum-free medium at 37°C for 48 hours in a humidified atmosphere of 5% CO2 in air. Neurite outgrowth was expressed as the length of the longest neurite from each DRG. The length of the longest neurite was measured with a square micrometer attached to an eyepiece; 11 to 20 DRGs were used for a single assay. In one experiment, ACK2 mAb (20 $\mu$g/ml) or anti-NGF mAb (250 ng/ml) was added to the culture 30 minutes before SCF.

**Assay of neuronal survival**

DRGs were dissected from mouse embryos at 15.5 day p.c. The DRGs from embryos of the same genotype were pooled and incubated in $\text{Ca}^{2+}/\text{Mg}^{2+}$-free Hank’s solution containing 0.1% trypsin (Sigma) and 0.002% DNase (Sigma) for 15 minutes at 37°C. The DRGs were then washed three times with the serum-free culture medium supplemented with soybean trypsin inhibitor (100 $\mu$g/ml, Sigma). Single cell suspensions were prepared by gentle pipetting and the dissociated cells were plated on 8-chamber culture slides (Nunc Inc., Naperville, IL) coated with poly-L-lysine (Sigma). The plating density was 4.0-5.7x10$^{4}$ cells per well. When comparisons were made among DRG neurons obtained from individual embryos, the cell density was adjusted to 4.0x10$^{4}$ cells per well. The plated DRG cells were cultured in the serum-free culture medium at 37°C in a humidified atmosphere of 5% CO2 in air. Surviving neurons were counted under a phase-contrast microscope using a square micrometer attached to an eyepiece. Neurons were identified as phase-bright round cells with a large nucleus.

**Detection of cells in S phase**

Dissociated DRG cells were incubated with culture medium containing 300 ng/ml BrdU (Wako Pure Chemical Ind, Osaka, Japan) for 48 hours at 37°C. The cultures were fixed with 70% ethanol. Cells that incorporated BrdU were visualized by staining with mouse anti-BrdU mAb (1:300, Becton Dickson, Mountain View, CA), biotinylated horse anti-mouse IgG antibody (1:300, Vector Laboratories) and streptavidin-biotin-peroxidase complex (1:200, Vector Laboratories). The binding of streptavidin-biotin-peroxidase complex was visualized with diaminobenzidine.

**RESULTS**

**Expression of $c$-kit receptors in DRGs**

First, we confirmed the expression of $c$-kit receptor in the DRGs of normal (C57BL/6-+/+) mouse embryos at 15.5 day p.c. Frozen DRG sections were stained with the ACK2 mAb that specifically bound the extracellular domain of the $c$-kit receptor (Nishikawa et al., 1991). About 10% of cells in each DRG were $c$-kit-positive, and they were considered to be neurons due to the presence of long processes (Fig. 1). DRGs of different segmental levels showed a similar distribution pattern of $c$-kit-positive neurons. Both central and peripheral axons expressed $c$-kit receptors.

We then examined whether the expression of $c$-kit receptor was abnormal in embryos of various mutant genotypes. In this experiment, embryos at 18.5 day p.c. were used because determination of their genotypes was easier than at 15.5 day p.c. The numbers of $c$-kit-positive cells and the total were not affected by the genotype of the embryos.
A normal number of c-kit-positive cells were counted in DRGs of C57BL/6-W42/W42 embryos regardless of the complete abrogation of the c-kit kinase activity (Table 1). Moreover, normal numbers of c-kit-positive cells were observed in embryos of WB-Sl/Sl and C57BL/6-Id/Id despite the lack of normal SCF (Table 1). Axons from DRGs of C57BL/6-W42/W42, WB-Id/Id and C57BL/6-Id/Id embryos expressed c-kit receptors normally (data not shown).

### Induction of neurite outgrowth by SCF

DRGs were dissected from C57BL/6-+/+ mouse embryos at 15.5 day p.c. and cultured in the serum-free medium with or without rmSCF. Non-neuronal fibroblast-like cells migrated from the explant and formed an area surrounding each DRG regardless of the presence or absence of rmSCF. When DRGs were cultured in the presence of rmSCF (100 ng/ml), numerous fine neurites grew beyond the area of fibroblast-like cells and directly attached to the plastic substrata of culture dishes (Fig. 2A). Most outgrowing neurites were stained with ACK2 mAb (Fig. 2B). In contrast, when DRGs were cultured in the absence of rmSCF, most outgrowing neurites remained within the area of fibroblast-like cells (Fig. 2C). None of outgrowing neurites were stained with ACK2 mAb (Fig. 2D).

The magnitude of neurite outgrowth was dependent on the concentration of rmSCF (Fig. 3). Neurites positive for c-kit were detectable with 1 ng/ml of rmSCF; neurite outgrowth at a half-maximal level was observed with 10 ng/ml and at a maximal level with 100 ng/ml. The ACK2 anti-c-kit mAb completely abrogated the rmSCF-dependent outgrowth of neurites (Fig. 3).

NGF induces the outgrowth of neurites from DRGs of mouse embryos (Thoenen and Barde, 1980). We compared the effect of rmSCF on neurite outgrowth to that of NGF. The magnitude of NGF-induced neurite outgrowth as determined by the length of neurites was comparable to that of rmSCF-induced neurite outgrowth. However, the number of NGF-dependent neurites was several times as great as the number of rmSCF-dependent neurites (Fig. 4). NGF-dependent neurites were tightly fasciculated and some of the neurites were stained with ACK2 mAb (data not shown). Although anti-NGF mAb abolished the NGF-dependent outgrowth of neurites, the addition of the same concentration of anti-NGF mAb did not affect the rmSCF-dependent neurite outgrowth (Fig. 3).

We then used mutant embryos of WW, W42/W42 and Sl/Id genotypes to confirm the effect of rmSCF on neurite outgrowth. The genetic background of WW is WB, whereas that of W42/W42 and Sl/Id is C57BL/6. However, when we compared DRGs of C57BL/6+/+ and WW embryos, neurite outgrowth from both gave comparable results either with or without rmSCF (data not shown).

rmSCF did not induce significant outgrowth of neurites from DRGs dissected from WW embryos that completely lack cell surface expression of the c-kit receptor (Nocka et al., 1990; Reith et al., 1990) or from DRGs of C57BL/6-W42/W42 embryos that lack the c-kit kinase activity (Table 2). WW-WW and C57BL/6-W42/W42 mouse embryos are severely anemic due to hypoproduction of erythrocytes and die in utero or shortly after birth. Failure of

### Table 1. Proportion of c-kit-positive cells in histological sections of DRGs from normal and mutant embryos

<table>
<thead>
<tr>
<th>Embryos</th>
<th>Embryo no.</th>
<th>No. of c-kit-positive cells per 10^3 cm^2</th>
<th>Total</th>
<th>No. of c-kit-positive cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6+/+</td>
<td>1</td>
<td>28±1</td>
<td>276±12</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30±2</td>
<td>309±15</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>29±3</td>
<td>350±30</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>35±3</td>
<td>359±16</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>32±1</td>
<td>341±8</td>
<td>9.4</td>
</tr>
<tr>
<td>C57BL/6-W42/W42</td>
<td>1</td>
<td>28±1</td>
<td>305±17</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>32±2</td>
<td>327±25</td>
<td>9.8</td>
</tr>
<tr>
<td>WB-Sl/Id</td>
<td>1</td>
<td>29±1</td>
<td>329±11</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>29±1</td>
<td>319±13</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>33±2</td>
<td>310±8</td>
<td>10.6</td>
</tr>
<tr>
<td>C57BL/6-Id/Id</td>
<td>1</td>
<td>32±2</td>
<td>347±12</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30±2</td>
<td>305±20</td>
<td>9.8</td>
</tr>
</tbody>
</table>

*Data from embryos of 18.5 day p.c. Means±s.e.m. of 4 microscopic fields (10^3 cm^2) from individual embryos.
neurite outgrowth from DRGs of WB-W/W and C57BL/6-W^{42}/W^{42} embryos was not attributed to the poor health of these embryos. In fact, rmSCF induced normal outgrowth of neurites from DRGs of C57BL/6-S^{41}/S^{41} embryos that was phenotypically similar to WB-W/W and C57BL-W^{42}/W^{42} embryos (Table 2).

**Trophic effect of SCF on DRG neurons**

It is possible that the outgrowth of c-kit-positive neurites resulted from the survival of DRG neurons induced by rmSCF. Since quantitation of neuronal survival is difficult in organ cultures of DRGs, those from 15.5-day p.c. embryos were dissociated into single cells by trypsinization. These were cultured and the number of surviving neurons was counted. When DRG cells were cultured without any additional factors, most of neurons died within the first 48 hours. In the presence of rmSCF, however, a significant number of neurons survived. The number of neurons started to decrease 4 days after the initiation of the culture even in the presence of rmSCF (data not shown). Thereafter, non-neuronal fibroblast-like cells vigorously proliferated, replacing the neurons. When the number of surviving neurons counted at 48 hours of the culture was used as an index of the trophic effect of rmSCF, we found that it was dependent on the rmSCF concentration. The effect was significant at 1 ng/ml and maximal at 100 to 300 ng/ml (Fig. 3). These values were comparable to the concentrations of rmSCF that were necessary for the neurite outgrowth from DRGs as shown in Fig. 3.

To rule out the possibility that rmSCF increased DRG neurons by promoting proliferation of neuronal precursor cells, DRG cells were cultured in medium containing BrdU, and cells in S phase of the cell cycle were assessed by BrdU incorporation. None of 143 neurons examined incorporated BrdU, but a large number of non-neuronal cells incorporated BrdU. The addition of rmSCF did not significantly affect the proportion of non-neuronal cells that incorporated BrdU; 75% of non-neuronal cells incorporated it in the presence of rmSCF and 78% did so in its absence.

NGF supports the survival of DRG neurons (Thoenen and Barde, 1980). The neurotropic effect of rmSCF was compared to that of NGF at saturation levels; rmSCF (300 ng/ml) and NGF (50 ng/ml). The number of neurons supported by NGF was 7.2 times greater than that supported...
Neurite outgrowth and neuronal survival by SCF

Moreover, adding of rmSCF to NGF did not increase the number of surviving neurons (Table 3). When neurons whose survival was supported by rmSCF or NGF alone or rmSCF + NGF were stained with ACK2 mAb, 86% of them supported by rmSCF alone were c-kit-positive whereas only 12% of them supported by NGF alone and 13% by rmSCF + NGF were c-kit-positive. In other words, the total number of surviving neurons was much greater when the culture was supported by NGF than by rmSCF, but no significant difference was observed in the number of c-kit-positive neurons between these two conditions. Moreover, rmSCF did not increase the number of c-kit-positive neurons when SCF was added with NGF.

We used mutant embryos of WB-W/W, C57BL/6-W42/W42 and WB-Sl/Sl to confirm the effect of rmSCF and NGF on the survival of DRG neurons. rmSCF did not support the survival of DRG neurons from WB-W/W and C57BL/6-W42/W42 embryos. In contrast, rmSCF supported the survival of DRG neurons from WB-Sl/Sl embryos that were phenotypically similar to WB-W/W and C57BL/6-W42/W42 embryos (Table 4). NGF supported the survival of DRG neurons from all mutant embryos examined (Table 4). Moreover, NGF supported the development of c-kit-positive neurons from DRGs of C57BL/6-W42/W42 and WB-Sl/Sl embryos as well as DRGs from C57BL/6-+/+ embryos (Table 4).

**DISCUSSION**

rmSCF induced the outgrowth of c-kit-positive neurites from DRGs of mouse embryos. Moreover, rmSCF

### Fig. 4. Outgrowth of neurites induced by either rmSCF or NGF.

DRGs of a C57BL/6-+/+ mouse embryo were cultured in the presence of (A) 100 ng/ml of rmSCF or (B) 50 ng/ml of NGF. Bar, 200 µm.

### Table 2. Effect of embryo genotypes on neurite outgrowth induced by rmSCF (100 µg/ml)

<table>
<thead>
<tr>
<th>Genotype of embryos</th>
<th>Neurite outgrowth (µm)*</th>
<th>Genotype of embryos</th>
<th>Neurite outgrowth (µm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo no.</td>
<td>rmSCF (−)</td>
<td>rmSCF (+)</td>
<td>Embryo no.</td>
</tr>
<tr>
<td>++/†</td>
<td>1</td>
<td>400±20</td>
<td>W/W</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>500±40</td>
<td>1110±70‡</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>370±40</td>
<td>1240±160‡</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>600±50</td>
<td>1360±180‡</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>490±20</td>
<td>1150±80‡</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>440±20</td>
<td>910±60‡</td>
</tr>
<tr>
<td>W42/W42</td>
<td>1</td>
<td>550±20</td>
<td>1130±150‡</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>500±30</td>
<td>540±30</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>420±30</td>
<td>490±30</td>
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<td>4</td>
<td>390±30</td>
<td>430±40</td>
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<td>5</td>
<td>520±30</td>
<td>420±20</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>600±50</td>
<td>580±70</td>
</tr>
</tbody>
</table>

*Means±s.e.m. of 11 to 17 DRGs from individual embryos.
†Data from C57BL/6-+/+ embryos.
‡P<0.01 by the t-test when compared with values in the absence of rmSCF.

### Fig. 5. Neuronal survival at various concentrations of rmSCF.

The number of total neurons (closed circles) and that of c-kit-positive neurons (open circles) were counted 48 hours after the initiation of the culture. Each point represents the mean of 9 microscopic fields (10⁻² cm² each) and vertical bars indicate the s.e.m.

by rmSCF (Table 3). Moreover, adding of rmSCF to NGF did not increase the number of surviving neurons (Table 3).
NGF (Thoenen and Barde, 1980), brain-derived neurotrophic factor (Lindsay et al., 1985; Davies et al., 1986), and S-100 protein (Chalazonitis et al., 1991) all promote both neurite outgrowth and neuronal survival. The present result added another example to the association of these two functions.

The action of rmSCF was dose-dependent, and anti-c-kit mAb abolished the effect of rmSCF on neurite outgrowth. The rmSCF did not induce neurite outgrowth even from DRGs of WB-/- embryos, indicating that the effect of NGF on c-kit-positive neurons is not mediated through endogenous SCF. Since the number of c-kit-positive neurons was comparable between cultures supported by NGF alone and by NGF+rmSCF, the c-kit-positive neurons appeared to be a subpopulation of NGF-responsive neurons.

The effect of rmSCF was observed at a concentration as low as 1 ng/ml and reached the maximal level at 100 to 300 ng/ml. These values are comparable with the effective concentrations of recombinant SCF for the proliferation of mast cells (Tsai et al., 1991) and for the survival of primordial germ cells (Godin et al., 1991; Dolci et al., 1991; Matsui et al., 1991). This implied that the effect of SCF on DRGs is physiological. There is evidence that axons are guided by diffusible chemotactants or immobilized cues to reach their targets (Dodd and Jessell, 1988). SCF appears to be expressed within DRGs (Keshet et al., 1991; Motro et al., 1991). Moreover, SCF is expressed by epithelial and mesenchymal cells in the skin (Matsui et al., 1990; Keshet et al., 1991), the peripheral targets of some DRG neurons, and by motor neurons in the spinal cord (Keshet et al., 1991), the central targets of some DRG neurons. SCF expressed by these cells may play a role in guiding axons from DRGs.

There is another possibility. In neural development, neurons are made in excess and a substantial number of them are eliminated during target innervation (Barde, 1989). This naturally occurring neuronal death is believed to have an important role in adjusting the number of neurons to the target size, thus ensuring accurate synaptic connections. From this perspective, the peripheral nervous system is one of the most studied system because of the early identification of NGF. Several lines of evidence have indicated that limiting amounts of neurotrophic factors are produced and support the survival of limited numbers of DRG neurons (Barde, 1989). SCF may have a trophic role for DRG neurons such as that seen in mast cells (Tsai et al., 1991).
and germ cells (Godin et al., 1991; Dolci et al, 1991; Matsu et al., 1991). A recent study of the murine cerebellum showed that c-kit-positive neurons internalized the soluble form of SCF and accumulated the SCF in perikarya by retrograde translocation (Manova et al., 1992). This suggests that SCF to c-kit signaling is indeed working in the nervous system. Moreover, the observation supports the neurotrophic role of SCF because uptake by nerve terminals and the subsequent retrograde transportation to neuronal perikarya are the common pathway for neurotrophic factors (Thoenen and Barde, 1980; Hendry et al., 1992).

To our knowledge, abnormalities suggesting defects in neuronal development have not been reported in homozygous or double heterozygous W or Sl mutant mice. The absence of any obvious neurological defects might be explained by the present results, which show most SCF-dependent neurons are also NGF-dependent. Alternatively, small defects may have escaped detection. Since c-kit-positive neurons represent a minor subpopulation of DRG neurons, SCF to c-kit signaling might have a role in the selective aspect of neural development. Further careful examination using homozygous or double heterozygous c-kit mutants of mice (Silvers, 1979; Russell, 1979) and rats (Niwa et al., 1991) may reveal abnormalities in neuronal development.

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