Incidence and Distribution of Experimental Metastases in Mutant Mice with Defective Organ Microenvironments (Genotypes \(SI/\text{Sl}^d\) and \(W/W^w\))

Francisco Arguello, Richard W. Furlanetto, Raymond B. Baggs, Brian T. Graves, Shari E. Harwell, Harvey J. Cohen, and Christopher N. Frantz

**ABSTRACT**

Mice carrying mutations at the \(SI\) (steel) and \(W\) (dominant white spotting) loci develop abnormalities on 3 embryonic migratory stem cell populations: hematopoietic stem cells, neural crest-derived melanocytes, and primordial germ cells. Transplantation experiments have indicated that the \(SI\) locus affects the microenvironment where stem cells migrate, proliferate, and differentiate, while the \(W\) locus affects the migratory cells themselves. The \(SI\) locus encodes for a multipotent growth factor known as stem cell factor. The \(W\) locus encodes the c-kit protein tyrosine kinase receptor whose ligand is the stem cell factor. We have investigated the incidence and organ distribution of experimental metastases after systemic intra-arterial injection of \(B16-G3.26\) melanoma cells into mutant \(SI/\text{Sl}^d\) and \(W/W^w\) mice. Both mutant mouse strains had a markedly lower incidence of ovarian metastases when compared with their congenic \(+/+\) mice. In contrast to the rare colonization of the ovaries, \(SI/\text{Sl}^d\) and \(W/W^w\) mice developed metastases in the myocardium, kidney, and stomach—anatomic sites that were infrequently or never affected in their congenic nonmutant mice. The only organs in which the average number of metastatic colonies differed between \(SI/\text{Sl}^d\) and \(W/W^w\) mice were the bone marrow and kidneys. The average number of colonized bones per mouse in the \(SI/\text{Sl}^d\) group was 5.0 ± 3.1 (SD), compared with 12.7 ± 5.3 in the \(W/W^w\) group. The average number of metastatic nodules in the kidneys of \(SI/\text{Sl}^d\) mice was 24.6 ± 9, while \(W/W^w\) mice had 15.5 ± 2.5. Mutant mice with multiple metastatic nodules in the kidneys, heart, and stomach were also found to have forestomach papillomas, an enlarged duodenum, kidney abnormalities, and small body size. The results of this study provide useful information on potential mechanisms of interaction of metastatic cells with their target organs, and suggest that there are additional organ defects associated with the mutations in the \(SI\) and \(W\) loci. They also document the importance of mutant mice in metastasis research.

**INTRODUCTION**

The propensity of certain types of tumors to preferentially metastasize to specific organs is determined by a number of factors, including intrinsic properties of specific tumor cell types, and anatomical aspects of the vasculature affected by the tumor, and the microenvironment provided by the target organs (1, 2). This organ microenvironment is conceptualized as a complex of extracellular matrix adhesive proteins and diffusible factors important in the lodging, invasion, and growth of metastatic cells. Some protein/peptide growth factors, such as IGF-I and IGF-II (3), epidermal growth factor (4), PDGF (5), granulocyte-colony stimulating factor, and granulocyte-macrophage colony-stimulating factor (6), have been shown to promote the in vitro growth of certain types of cancer cells, and it is possible that they play a role in organ specificity of metastases, since some of these cytokines are tissue specific or present in increased amounts in certain organs.

The use of mutant mice with specific microenvironmental defects in certain tissues may provide a research tool to investigate interactions of metastatic cells with their target organs. Mice bearing the mutations designated \(SI/\text{Sl}^d\) (steel-dickie) and \(W/W^w\) (dominant white spotting-viable) are characterized by being black-eyed with white hair, sterile, and severely anemic—the underlying mechanisms are different. Transplantation experiments with neural crest, skin, and bone marrow (for review, see Ref. 6) have indicated that the defects in \(SI/\text{Sl}^d\) mice reside in the organ microenvironment in which precursor cells lodge, grow, and differentiate into blood cells (bone marrow), melanocytes (skin), and germ cells (ovary/testis). Conversely, the depletion of erythrocytes, melanocytes, and germ cells in the \(W/W^w\) mice is due to an intrinsic defect in the precursor cells that migrate to the affected organs. Over the past 2 years, the defects of \(SI/\text{Sl}^d\) and \(W/W^w\) mutant mice have been identified at the molecular level. The \(SI\) locus, located on chromosome 10 in the mouse, encodes a peptide growth factor known as “mast cell factor” or SCF (9–15). SCF is present in 2 forms—membrane bound and soluble protein (11)—and both forms are active on a number of target cell types, including early and late hematopoietic progenitor cells, mast cells, melanoblasts, and primordial germ cells (16). In contrast, the \(W\) locus, located on chromosome 5 in the mouse, contains the proto-oncogene c-kit that encodes a transmembrane tyrosine kinase receptor (17, 18) whose ligand is SCF (19). The c-kit receptor-SCF ligand complex appears to provide a mechanism for homing, survival, proliferation, and differentiation of migratory stem cells during early and late development (20–23). In \(SI/\text{Sl}^d\) mice, the \(SI\) allele is completely deleted for coding sequences of the growth factor (12), while \(SI^d\) has the potential to express wild-type soluble factor but not its membrane bound form (24, 25). In \(W/W^w\) mice, \(W\) allele is deleted for the transmembrane domain of the c-kit receptor and functions as a null mutation, while \(W^w\) contains a missense mutation in the kinase domain of the receptor resulting in impaired activity, and exhibits a dominant negative phenotype (26). Consequently, the absence of SCF in its membrane bound form, or the interference with wild-type c-kit mediated signal transduction by the \(W^w\) mutant receptor, account for the depletion of embryonic migratory stem cell populations in these mutant mice.

The current study was undertaken to determine whether the inability of the bone marrow to support hematopoietic stem cells, or the ovaries to support primordial germ cells in the \(SI/\text{Sl}^d\) mice, could also be reflected as inability to support the metastatic colonization of those organs by cancer cells whose normal counterpart cells are natural targets for SCF. We have
studied the incidence and organ distribution of experimental metastases in Sl/Sld mutant mice, and in their normal congenic +/+ counterparts after injection of B16 melanoma cells. We also studied the distribution of experimental metastases in the W/Wv mutant mice and their congenic +/+ mice to be used as comparative controls. We chose the B16-G3.26 melanoma clone because of its melanoblast-derived origin, histocompatibility with the mutant mouse strains, and marked propensity to colonize the bone marrow and ovaries of normal syngeneic mice after arterial injection (27). The cells were injected into the systemic arterial circulation (left ventricle of the heart) to provide vascular access to all organs (2). A marked difference in the incidence of ovarian metastasis between mutant and normal congenic mice led us to perform additional studies to determine whether constitutive hormone production by the ovarian follicles is involved in the pathogenesis of ovarian metastasis. The results of these studies are described.

MATERIALS AND METHODS

Animals. Female mutant WCB6Fr/J-SI/Sld and WBB6Fr/J-W/Wv mice, and their normal congenic WCB6Fr/+/+ and WBB6Fr/+/+ mice, respectively, were supplied by The Jackson Laboratory (Bar Harbor, ME). Female C57BL/6 mice were purchased from Charles River (Wilmington, MA). The animals were 8 to 10 weeks old and were maintained under the guidelines established by the NIH and the University of Rochester.

Cell Line. The G3.26 subline of B16 murine melanoma (28) was generously provided by Dr. C. Stackpole. The B16-G3.26 cells colonize almost exclusively the lungs after i.v. injection, and the adrenal glands, bone/bone marrow, and ovaries when injected into the systemic arterial circulation (2). The cells were grown and harvested as described previously (2, 27). Cell viability was determined by trypsin blue exclusion, and only cell suspensions with greater than 90% viability and without cell clumping were used.

SIA. Using a modification of our previously described technique (2, 27), we anesthetized the animals with i.p. injections of sodium pentobarbital (60–90 mg/kg). The anterior chest wall was then scrubbed with barbital (60–90 mg/kg). The anterior chest wall was then scrubbed with barbital (60–90 mg/kg). The anterior chest wall was then scrubbed with barbital (60–90 mg/kg). The anterior chest wall was then scrubbed with barbital (60–90 mg/kg). The air bubble in the plunger side of the syringe before injection (Fig. 1). Cells were injected slowly over 20 to 40 s. A new syringe and needle were used for each injection. Unless otherwise specified, 10⁴ cells were injected into each animal. The mice were sacrificed 16 to 18 days after tumor cell injection.

Ovarian Suppression. To investigate the role of gonadotropins in stimulating follicular-ovarian growth factor production, gonadotropin secretion was inhibited using a long acting preparation of leuprolide acetate (Lupron Depot; TAP Pharmaceuticals, North Chicago, IL). Leuprolide acetate, a GnRH0, acts as a potent inhibitor of gonadotropin secretion by desensitizing the pituitary after continuous exposure to the agent (29). Female C57BL/6 mice were treated with either 170 mg or 500 mg GnRH0 i.m. for 2 weeks and 1 week prior to the injection of the B16 melanoma cells. Control mice received the diluent. To verify that GnRH treatment was effective, in each experiment 2 or 4 treated and nontreated mice were sacrificed 2 days before the scheduled day for tumor cell injection, and the uterine horns and ovaries were removed, weighed, and examined histologically.

Determination of Metastasis in Specific Organs. Pigmented melanotic colonies, when present, were easily seen on the surface of the organs or in the medullary cavities of the bones and counted as described previously (27). Metastases to the ovaries were evaluated in the following ways: (a) number of mice with colonized ovaries per total number of mice; and (b) number of metastasized ovaries per total number of ovaries. Histological examination was performed using standard techniques of paraffin embedding, sectioning, and staining.

Statistical Analysis. The statistical significance of differences between groups was analyzed using the Student’s t test.

RESULTS

Organ Distribution of Experimental Metastasis in Mutant SI/ Sld and Normal Congenic +/+ Mice. The incidence of metastases between SI/Sld and their control +/+ mice differed in the ovaries, myocardium, kidneys, stomach, and bone/bone marrow. Table 1 summarizes the pooled results of 3 experiments on the incidence and organ colonization pattern of metastases after SIA injection of B16-G3.26 melanoma cells.

Table 1: Organ distribution of metastasis after SIA injection of B16 melanoma cells into SI/Sld, W/Wv, and congenic +/+ mice

<table>
<thead>
<tr>
<th>Organ</th>
<th>No. of mice with tumor/no. of mice given injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI/Sld</td>
<td>W/Wv +/+</td>
</tr>
<tr>
<td>Ovaries</td>
<td>1/15 14/14 5/14 15/16</td>
</tr>
<tr>
<td>Uterus</td>
<td>0/15 2/14 3/14 2/14</td>
</tr>
<tr>
<td>Bone/marrow</td>
<td>14/15 (5.0) 14/14 (8.5) 14/14 (12.7) 16/16 (9.0)</td>
</tr>
<tr>
<td>Adrenal</td>
<td>13/15 14/14 12/14 13/16</td>
</tr>
<tr>
<td>Kidneys</td>
<td>6/15 (24.0) 5/14 3/14 (15.5) 1/16 (1.0)</td>
</tr>
<tr>
<td>Stomach</td>
<td>4/15 (3.0) 5/14 2/14 (3.0) 0/16</td>
</tr>
<tr>
<td>Myocardium</td>
<td>10/15 (2.4) 14/14 (1.0) 10/14 (9.5) 2/14 (1.0)</td>
</tr>
<tr>
<td>Liver</td>
<td>0/15 0/14 0/14 0/16</td>
</tr>
<tr>
<td>Brain</td>
<td>1/15 0/14 0/14 0/16</td>
</tr>
<tr>
<td>Lungs</td>
<td>4/15 (2.4) 14/14 (1.2) 2/14 (2.0) 0/16</td>
</tr>
<tr>
<td>Skin</td>
<td>0/15 1/14 0/14 0/14</td>
</tr>
<tr>
<td>Spleen</td>
<td>0/15 0/14 0/14 0/14</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0/15 0/14 2/14 0/16</td>
</tr>
<tr>
<td>Bladder</td>
<td>0/15 0/14 0/14 0/14</td>
</tr>
<tr>
<td>Mesentery</td>
<td>3/15 3/14 2/14 2/16</td>
</tr>
</tbody>
</table>

* Average number of colonized bones per mouse.

A average number of colonies per organ.

red oxygenated blood into the transparent needle hub during the entire procedure, indicating proper positioning of the needle into the left ventricle, was greatly facilitated by creating and leaving a small air bubble in the plunger side of the syringe before injection (Fig. 1). Cells were injected slowly over 20 to 40 s. A new syringe and needle were used for each injection. Unless otherwise specified, 10⁴ cells were injected into each animal. The mice were sacrificed 16 to 18 days after tumor cell injection.
Fig. 2. Organ distribution of experimental metastases. a. Lungs (LU), heart (HE), stomach (ST), adrenal glands (AD), kidneys (KI), ovaries (OV), and uterine horns (UH) from a congenic +/+ mouse. Adrenal glands and ovaries appear replaced by pigmented melanoma tumor. b. Same organs as those described above, but from a SI/SI' mouse. The heart appears enlarged and with intramyocardial metastatic nodules (arrows). The adrenal glands are replaced by tumor. The ovaries and uterine horns appear atrophic and free of metastatic disease. c. Same organs as those shown in a and b, but from a SI/SI' mouse with runty appearance. The heart appears enlarged and with multiple metastatic nodules in the myocardium. Small pigmented colonies are also seen in the lungs. The stomach cavity is small with a thick wall and irregular luminal surface, due to the presence of papillomas and pigmented metastatic nodules. One adrenal gland appears colonized, and the kidneys have multiple melanotic colonies. The ovaries and uterine horns appear atrophic and free of metastatic disease.

Table 2 Incidence of metastasis in the ovaries after SIA injection of B16 melanoma cells into SL/SL', W/W', and congenic +/+ mice

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Incidence of ovarian tumor colonization</th>
<th>No. of metastasized ovaries/no. of ovaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>14/14 (100%)</td>
<td>26/28 (92.8)</td>
</tr>
<tr>
<td>SI/SI'</td>
<td>1/15 (6.6)</td>
<td>1/30 (3.3)</td>
</tr>
<tr>
<td>+/+</td>
<td>15/16 (93.7)</td>
<td>27/32 (84.3)</td>
</tr>
<tr>
<td>W/W'</td>
<td>5/14 (35.7)</td>
<td>6/28 (21.4)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percent incidence.

Fig. 3. Histological features of a metastatic nodule (ME) in the stomach of a SI/SI' mouse, which appears surrounded by hyperplastic papilliform keratoic epithelium (PP).
**EXPERIMENTAL METASTASIS IN SI/SI* AND W/W* MICE**

Fig. 4. Representative histological sections of the kidney of congenic +/+ and SI/SI* mice. a. Fine structure of a normal glomerulus (G) and tubules from a normal congenic +/- mouse. T, capillary tuft; B, Bowman's space. b. Glomeruli from a kidney of a SI/SI* mouse showing some increase in cellularity, with rare adhesions of visceral to parietal Bowman's capsule. c. A glomerulus in the kidney of a SI/SI* mouse with forestomach papillomas showing increased cellularity, excessive mesangial matrix, and complete obliteration of Bowman's space.

SI* mice (5.0 ± 3.1) when compared with their normal +/+ counterparts (8.5 ± 3.0; P < 0.005).

Organ Distribution of Experimental Metastasis in Mutant W/W* and Congenic +/+ Mice. Table 1 also summarizes the pooled results of 3 experiments on the organ colonization pattern of experimental metastases in the mutant W/W* mice and congenic control +/+ mice after SIA injection of B16-G3.26 melanoma cells. As observed with SI/SI* mice, there was a significantly lower incidence of ovarian metastasis in W/W* mice when compared to their congenic +/- control mice. While 15 of 16 +/- control mice developed massive replacement of the ovaries, only 5 of 14 W/W* mice were found to have small tumor masses in the ovaries, and in only one instance it was bilateral (Table 2). Histological examination of ovaries from W/W* mice also revealed the absence of ovarian follicles. As in the case of SI/SI* mice, the hearts of W/W* mice were frequently found to have multiple small metastatic nodules located predominantly in the right ventricles (10 of 14 mice). As found in 4 SI/SI* mice, 3 W/W* mice had metastatic nodules in their stomachs coexisting with forestomach papillomas. Two mice with metastatic nodules in the stomachs were the only animals with metastatic nodules in the kidneys and a runty appearance. However, the metastatic nodules in the kidneys were fewer and smaller than those seen in the SI/SI* mice (Table 1). In contrast to SI/SI* mice, W/W* mice had a slightly increased average number of colonized bones per mouse (12.7 ± 5.3) when compared with their control +/- mice (9.0 ± 3.8; P < 0.03).

Incidence of Ovarian Metastasis in Mice with Luprolide-suppressed Ovarian Function. Both SI/SI* and W/W* mutant mice have a complete absence of ovarian follicles, and both showed a markedly low incidence of ovarian metastasis. These observations suggested that growth factor/hormone production by the ovarian follicles might be critical for the successful colonization of normal ovaries by the B16 melanoma cells. To test this hypothesis, we suppressed ovarian follicular function of C57BL/6 mice by inhibiting pituitary gonadotropin secretion with a long acting preparation of leuprolide acetate as described in “Materials and Methods.” We documented ovarian suppression in the GnRHa-treated mice by the reduction in more than 50% of the normal weight of the ovaries and uteri, and histological evidence of atrophy of the ovarian follicles and uterine horns. The results of 3 different experiments are summarized in Table 3. We found a slight, but consistent, decrease in the number of colonized ovaries in the GnRHa-treated groups as compared with the nontreated control groups. This difference between groups was statistically significant (P < 0.05) when the results of all 3 experiments were compared. The incidence and distribution of metastases in other organs were not affected by the GnRHa treatment.

**DISCUSSION**

Mutant SI/SI* and W/W* mice were found to have a markedly lower incidence of ovarian metastasis compared to normal congenic mice. During normal embryogenesis, primordial germ cells migrate from the yolk sac splanchnopleura to the germinal ridges (30). When the supply of SCF is defective (as in SI/SI* mice), the ovarian follicles undergo atrophy, and the absence of ovarian follicles results in the low incidence of ovarian metastasis. This observation suggests that ovarian follicles produce a growth factor/hormone that is critical for the successful colonization of normal ovaries by the B16 melanoma cells.

| Table 3 Incidence of ovarian metastasis in C57BL/6 mice pretreated with injections of GnRH analogue prior to SIA injections of B16-G3.26 melanoma cells |
|-----------------|-----------------|-----------------|
| GnRHa | No. of cells | Incidence of ovarian metastasis/no. of mice | No. of metastasized ovaries/no. of ovaries |
| 170 mg vehicle | 2.5 x 10^4 | 4/4 | 6/8 (75%) |
| 170 mg vehicle | 1.0 x 10^4 | 3/5 | 3/10 (30) |
| 500 mg vehicle | 2.5 x 10^4 | 9/10 | 14/20 (70) |

* Numbers in parentheses, percent incidence.
mice) or the ability to receive the signal is disabled (as in W/ W° mice), primordial germ cells fail to reach or survive in the gonads (8). Consequently, both Sl/SI° and W/W° female mice have a depletion or complete absence of ovarian follicles and sex steroids (estrogen and progesterone) and are hypogonadal with high level of gonadotropins in plasma (7, 8, 31). Human and murine melanoma cells are known to have functional receptors for estrogens (32, 33). In fact, estrogen treatment of animals promotes tumor growth and metastases by the B16 melanoma cells (34). It is therefore possible that the constitutive estrogen production by the ovarian follicles plays a role in the survival and growth of B16 melanoma cells seeding the ovaries, and the sparse ovarian colonization found in Sl/SI° and W/W° mice might be the result of the low levels or absence of estrogen in their ovarian microenvironment. This concept of endocrine-dependent metastatic growth in the ovaries appears to be supported by our results obtained in mice whose ovarian hormonal production was suppressed using a GnRH agonist; mice treated with the agonist had a lower incidence of ovarian colonization. However, hormonal suppression did not decrease ovarian metastasis to the very low incidence seen in the mutant mice. Also, hormonal suppression resulted in decreased ovary weight, which could result in turn in decreased metastasis.

Multiple metastatic nodules were found in the myocardium of both mutant mouse strains. Northern blot analysis of RNA isolated from embryonic mouse tissues and in situ hybridization techniques have shown that SCF (23) and c-kit transcripts (35) are present in organs associated with hematopoiesis. The defective c-kit receptor-SCF ligand system in SI/SI° and W/W° mice results in inefficient hematopoiesis, and consequently these mice are severely anemic despite higher than normal plasma erythropoietin levels (7). It is well known that anemia imposes an increased functional demand on the heart, whose adaptive response is hypertrophy (36). Molecular events associated with muscle hypertrophy involve increased production of PDGF (37), IGF-I, and IGF-II (38) by the myocytes. Since these growth factors stimulate the in vitro growth of certain types of cancer cells, including melanoma cells (5, 39), it seems possible that the increased number of melanoma nodules found in the myocardium of mutant mice could be the result of increased levels of PDGF and/or IGF in the cardiac microenvironment. However, the reason why the mutant mice develop metastatic nodules predominantly in the myocardium of the right ventricle is not clear, and suggests that other factors may also be involved (see below).

We have identified in both Sl/SI° and W/W° mice the occurrence of an intriguing metastatic syndrome characterized by the development of multiple metastatic nodules in the kidneys, increased number of metastases in the heart, and colonization of the stomach. Invariably, Sl/SI° mice with this metastatic triad were found to have forestomach papillomas, an abnormal duodenum, renal defects, and a reduced body size. The increased incidence of spontaneous forestomach papillomas in both mutant mouse strains (up to 40% of the animals) was previously recognized by Kitamura and colleagues (40, 41), who also noticed that these mice have a runty appearance. It is known that the development of skin papillomas results in the proliferation of normal melanocytes in these lesions (42), which suggests a paracrine growth effect of the papilloma cells on the melanocytes. Thus, it seems possible that a paracrine growth effect could also take place on melanoma cells seeding the stomachs of mice with forestomach papillomas, but the reason for the association with multiple metastatic nodules in kidneys and heart is not clear. However, unrecognized mutant defects in the digestive system, kidneys, and heart could be an explanation, particularly because the c-kit receptor-SCF ligand complex also appears to play a role in the embryogenesis of these 3 organs (20, 23, 35).

We found only a slight decrease in the average number of colonized bones per mouse in the Sl/SI° group and a slight increase in the average number of colonized bones in the W/W° mice when compared with their respective congenic controls. However, while there was no difference in the average number of colonized bones between the 2 congenic +/+ control groups (8.5 ± 3 versus 9.0 ± 3.8, P > 0.7), there was a statistically significant difference between the average number of colonized bones in the Sl/SI° compared to W/W° mice (5.0 ± 3.1 versus 12.7 ± 5.3, respectively; P < 0.00005). This 2-fold difference in the average number of colonized bones is equivalent to a 2-fold difference in number of tumor cells injected, according to our previously published data (27). Despite the fact that c-kit gene expression has been detected in the B16 melanoma cells (43), we have been unable to demonstrate any effect on the in vitro growth of our B16 clone with different concentrations (50, 100, and 200 ng/ml) of recombinant mouse SCF. In the bone marrow, SCF is believed to act synergistically with other hematopoietic growth factors during hematopoiesis. In fact, SCF does not act on stem cells in the absence of other hematopoietic factors (16, 44). It is possible that SCF in the presence of other growth factors, such as those produced in the bone marrow microenvironment (6), affect tumor cell growth. Nevertheless, it is not clear why W/W° mice develop more bone marrow tumor colonies than congenic nonmutant mice.

In conclusion, this study shows that the use of mutant mice with primary or secondary abnormalities in the microenvironment of specific organs is a valuable tool in metastasis research, and offers the opportunity to determine tissue factors that are important in the establishment of metastases in specific anatomical sites. The rare colonization of the ovaries found in Sl/SI° and W/W° mice, which lack functional ovaries, demonstrates that merely the presence of the organ or the clonogenic properties of the cancer cells is not sufficient to develop metastatic colonization, but the existence of a functional-inductive microenvironment in the target organ is also required. We have identified a subpopulation of mutant mice that have forestomach papillomas and a small body size, and that characteristically develop multiple metastatic B16 melanoma colonies in the kidneys, heart, and stomach. It is possible that the development of forestomach papillomas, the susceptibility of the kidneys and heart to be colonized by the B16 melanoma cells, and perhaps the small body size of these animals, are a reflection of additional organ abnormalities linked to the mutations in the SI and W loci. Finally, a role of SCF in the establishment of bone marrow metastasis was suggested, but not proved, in this study with melanoma cells. The use of either germ cell tumor cell lines or leukemia cell lines in these mice is an attractive alternative to study further the potential role in metastatic growth of the c-kit receptor-SCF ligand communication system of migratory cells.

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