Decreased c-kit function inhibits enhanced skin carcinogenesis in c-Ha-*ras* protooncogene transgenic mice

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We previously showed that rasH2 transgenic mice carrying the human c-Ha-*ras* protooncogene are highly susceptible to chemical skin carcinogenesis. In the dermis of rasH2 mice, mast cells are recruited constitutively, and the number of mast cells increases more than in wild-type mice in response to treatment with 7,12-dimethylbenz[a]anthracene and 12-*O*-tetradecanoylphorbol-13-acetate. To determine whether enhanced skin tumor development in rasH2 mice is dependent on the recruitment of mast cells, we generated rasH2 *KIT*$^{W/Wv}$ mice by crossing rasH2 mice and W or W^{v} *KIT* mutants, and examined the chemical skin carcinogenesis. In rasH2 *KIT*$^{W/Wv}$ mice, mast cells were not found in the dermis either before or after treatment with 7,12-dimethylbenz[a]anthracene and 12-*O*-tetradecanoylphorbol-13-acetate. Papilloma multiplicity was up to 4.6-fold higher in rasH2 *KIT*$^{+/+}$ mice compared with their rasH2 *KIT*$^{W/Wv}$ siblings. At 12 weeks after the experiment began, the volumes of tumors were significantly smaller in rasH2 *KIT*$^{W/Wv}$ relative to rasH2 *KIT*$^{+/+}$ mice (rasH2 *KIT*$^{W/Wv}$: 29.2 ± 19.9 mm^3 versus rasH2 *KIT*$^{+/+}$: 179.6 ± 726.6 mm^3; $P = 0.0153$). There was no difference in the latency or multiplicity of papillomas between mice without the rasH2 transgene, *KIT*$^{W/Wv}$ mice and their wild-type littermates. Western blot analysis showed that expression of H-RAS protein in the skin was equivalent in rasH2 *KIT*$^{W/Wv}$ and rasH2 *KIT*$^{+/+}$ mice. In conclusion, the inhibition of c-kit decreased H-ras-induced skin carcinogenesis. The suppression of c-kit may be a unique and effective target as a preclinical model of cancer treatment where the activation of H-ras has a significant role. Targeting mast cells could also be a potential strategy for treating malignancies. (*Cancer Sci* 2007; 98: 1549–1556)

Human cancers develop through a multistep process that involves the accumulation of genetic mutations.[1] Based on the results of work in experimental animal model systems, the carcinogenesis process can be divided into the initiation, promotion and progression stages.[2,3] The initiation stage is an irreversible event in which carcinogens damage DNA and induce mutations in critical genes in target stem cells. During the promotion stage, initiated cells undergo selective clonal expansion due to the acquisition of a proliferative advantage, or the ability to evade growth inhibitory or apoptotic signals. An example of this is the activating mutations of c-Ha-*ras*.[4] Three members of the RAS family – H-RAS, K-RAS and N-RAS – are activated by mutations in human tumors.[5] Almost all RAS activation in tumors is accounted for by mutations at codons 12, 13 and 61.[6] These mutations all compromise the GTPase activity of RAS, preventing GAP from promoting hydrolysis of the GTP binding to RAS and therefore causing RAS to accumulate in the GTP-bound, active form.

The mouse skin carcinogenesis model is one of the most well-defined *in vivo* models of experimental carcinogenesis. In DMBA-initiated mouse skin, repeated applications of the tumor promoter TPA promotes papillomas and eventually carcinomas.[7] DMBA induces a mutation at either codon 12 or 61 of the Ha-*ras* gene. Consequent addition of TPA has a promotion effect, activating Stat3.[8]

RasH2 mice carry the human c-Ha-*ras* protooncogene with its own promoter region,[9] and are highly susceptible to chemical carcinogenesis.[10–12] We previously reported that rasH2 mice had enhanced chemical skin carcinogenesis in response to treatment with DMBA and TPA.[9] In rasH2 mice, the number and total volume of papillomas were far greater than those of their wild-type littermates, presumably because of the increased abundance of initiated cells in rasH2 mice. Furthermore, the latency of the formation of both squamous cell papilloma and squamous cell carcinoma after treatment with DMBA was shorter in rasH2 mice. This shorter duration of latency in tumor development compared with wild-type mice suggests that the activated rasH2 gene functions as a tumor promoter.[9]

Ras oncogene expression promotes and sustains the tumor–host interactions that are essential for neoplastic development. Constitutive Ras activity has been shown to contribute to increased tumor cell invasiveness through the activation of matrix metalloproteinases.[13] During tumor progression, cancer cells recruit immune cells, which remodel the tumor stroma and initiate angiogenesis.[14–16] Indeed, mast cells are present at the periphery of both experimentally induced rodent tumors[17,18] and human neoplasms.[19–21] Coussens *et al*.[14] investigated the importance of mast cells as tumor promoters in the skin carcinogenesis of K14-HPV16 mice. They found that infiltrating mast cells turn on and progressively intensify angiogenesis by releasing sequestered angiogenic activators in the premalignant early phase of hyperplasia and dysplasia in K14-HPV16 mice. These workers generated a K14-HPV16 KIT^{W}/KIT^{Wv} mouse, which has a mast cell deficiency. Severe attenuation of early neoplasia is observed in these mice, which demonstrates the essential role of mast cells in the development of premalignant lesions.[14] However, the role of mast cells in late carcinogenesis events is not clear. In the current study, we generated rasH2 mice that were deficient in mast cells by crossing rasH2 and W and Wv *KIT* mutant mice, and conducted experiments using chemical skin carcinogenesis.

Materials and Methods

Animals. All animals were handled in accordance with the guidelines established by the Institute of Medical Science, The University of Tokyo, and Teikyo University (Tokyo, Japan). We used a rasH2 transgenic mouse line generated from mouse strain C57BL/6J as described previously.[22]

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Abbreviations: ABC, avidin–biotin–peroxidase complex; ATP, adenosine triphosphate; DMBA, 7,12-dimethylbenz[a]anthracene; GAP, GTPase-activating protein; GTP, guanosine triphosphate; GTPase, guanosine triphosphatase; HPV, human papillomavirus; PBS, phosphate-buffered saline; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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***KIT*^{W}/*KIT*^{Wv} genotyping.** *C57BL/6J-KIT*$^{W/+}$ (Jackson Laboratories, Bar Harbor, ME, USA) and *C57BL/6J-rasH2* mice were intercrossed to obtain mice with the rasH2 transgene and the *KIT*$^{W/+}$ genotype. These mice were then intercrossed with *C57BL/6J-KIT*$^{Wv/+}$ mice (Jackson Laboratories) to obtain mice with the rasH2 transgene with the *KIT*^{W}/^{Wv} genotype. The conventional W mutant of *KIT* is a deletion-type mutation lacking a 78-amino acid transmembrane portion of the receptor, so that no receptors are exposed on the cell surface. The W^{v} mutant of *KIT* has a single amino acid exchange, at position 660 (threonine to methionine), which coincides with the ATP-binding site of the receptor.[23]

This cross yielded human c-Ha-*ras* transgene-positive (rasH2) mice with the *KIT*$^{+/+}$ ($n = 10$), *KIT*$^{Wv/+}$ ($n = 7$), *KIT*$^{W/+}$ ($n = 4$) and *KIT*$^{W/Wv}$ ($n = 8$) phenotypes; and *KIT*$^{Wv/+}$ ($n = 10$), *KIT*$^{W/+}$ ($n = 10$), *KIT*$^{W/Wv}$ ($n = 4$), transgene-negative and *KIT*$^{+/+}$ ($n = 10$) mice, which were used as wild type. Genotypes and the presence of the transgene were determined from tail DNA by using a polymerase chain reaction-based assay described elsewhere.[9,24] All mice were bred under specific pathogen-free conditions. The animals were allowed free access to the CRF-1 powdered basal diet (Oriental Yeast, Tokyo, Japan) and to tap water.

Immunohistochemistry. For morphological evaluation, mouse tissues were fixed in 4% paraformaldehyde in PBS and embedded in paraffin. Sections (3–5 μm thick) were stained with hematoxylin–eosin in accordance with standard protocols. Morphological analysis was carried out using a BX51 Olympus microscope (Olympus, Tokyo, Japan). For immunohistochemistry, after deparaffinization through a graded xylene and ethanol series, sections were washed in PBS (pH 7.4) and treated with 0.3% hydrogen peroxide in PBS for 30 min. After incubation for 30 min with 10% normal goat serum to block non-specific binding of the antibodies, the sections were incubated with goat polyclonal anticyclin D1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After overnight incubation with the primary antibody at 4°C, the sections were reacted with biotinylated secondary antibody for 30 min. Subsequently, the sections were allowed to react for 30 min with ABC using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) and subjected to the peroxidase reaction with 0.02% 3,3′-diaminobenzidine tetrahydrochloride as a chromogen in PBS containing 0.007% hydrogen peroxide. All immunolocalization experiments were repeated three times on multiple tissue sections and included negative controls for determination of background staining, which was negligible.

Enzyme histochemistry. We examined the distribution of mast cells in the skin of rasH2 and wild-type littermates using chloroacetate esterase histochemistry and toluidine blue staining. Mast cells were visualized in 5-μm paraffin-embedded tissue sections that had been deparaffinized in xylene, hydrated through a graded alcohol series (100, 95, 70 and 50% ethanol) and equilibrated in H_2O. Chloroacetate esterase histochemistry was carried out as described previously to detect the chymotrypsin-like serine esterase activity of mast cells.[25,26] Cytochemical reaction mixtures (0.53 mM naphthol AS-D chloroacetate, 0.3 mM Fast blue BB salt, 500 mM NaF, 10% Triton X-100, 1 M Tris-HCl pH 6.8) were made fresh daily and were filtered through Whatman #1 paper (Whatman Inc., Florham Park, NJ, USA) immediately before use. After incubation in this solution at 30°C, slides were rinsed in water and counterstained. Slides were then washed extensively in water, counterstained in Gills Hematoxylin #3 (3 s), dehydrated in 100% alcohol, and mounted under coverslips in 100% glycerol. To identify the metachromatic granules of mast cells, 5-μm paraffin-embedded tissue sections were stained for 20 min in 1% toluidine blue (pH 4.1, in water), dehydrated in 100% alcohol and mounted under coverslips in 100% glycerol.

Carcinogenesis study. The procedure used to conduct the chemical skin carcinogenesis experiments has been described elsewhere.[9] Briefly, 8-week-old male mice were treated initially with 25 μg of DMBA (Sigma-Aldrich, St Louis, MO, USA) dissolved in 0.1 mL acetone, then painted onto shaved skin on the backs of the mice. After 1 week, 0.2 μM TPA (Sigma-Aldrich) dissolved in 0.1 mL acetone was applied twice per week for the following 20 weeks. Solutions were prepared every week and kept in light-shaded bottles. Daily observations were made for mortality and clinical signs. The number and volume of skin tumors were recorded at the time of TPA application and at the time the animals were killed. All mice were killed 25 weeks after DMBA treatment by cervical translocation. Skin tissues were excised from the subcutaneous tissue with scissors, along with the fat pads. Skin tumors and normal skin without any macroscopic abnormalities (negative controls) were cut in half. One piece of each tumor sample was fixed in formaldehyde then processed for paraffin embedding for histological examination. The remaining half-pieces were frozen immediately in liquid nitrogen for DNA extraction, for mutation analysis. For histopathological examination, paraffin-embedded back skin was sectioned to 4-mm thick, and stained with hematoxylin–eosin.

Expression of RAS protein in mouse skin. Proteins were prepared from the skin of 6-week-old male mice as described previously.[27] Proteins were separated by 12% sodium dodecylsulfate–polyacrylamide gel electrophoresis then transferred to an Immobilon membrane (Millipore, Billerica, MA, USA). Immunoblots were probed with polyclonal anti-H-*ras* (Santa Cruz Biotechnology) antibody and visualized by enhanced chemiluminescence (ECL-plus; Amersham Biosciences, Piscataway, NJ, USA).

Statistical analysis. Statistical testing included Student's *t*-test; all comparisons were two-tailed, and a *P*-value less than 0.05 was considered significant.

Results

Accumulation of mast cells in the dermis of rasH2 mice. Topical application of TPA causes inflammation in the dorsal skin of mice. Mast cells are essential for formation of this TPA-induced skin inflammation.[28] In wild-type mice, the density of mast cells in the dermis increased after treatment with DMBA and TPA. The density of mast cells also increased with a change from normal epidermis to hyperplasia and papilloma in the course of chemical carcinogenesis (Fig. 1). Figure 1 shows that

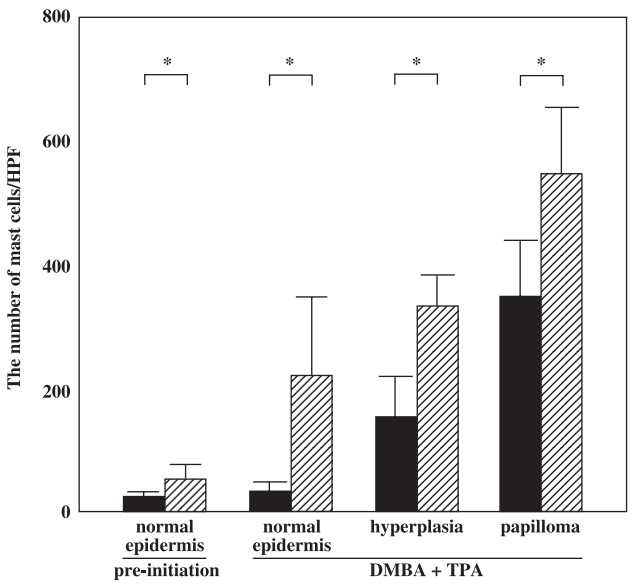

Fig. 1. The number of mast cells that accumulated in the dermis of treated skin was counted. (■), wild type; (▨), rasH2; HPF, high-power field. *$P < 0.05$.

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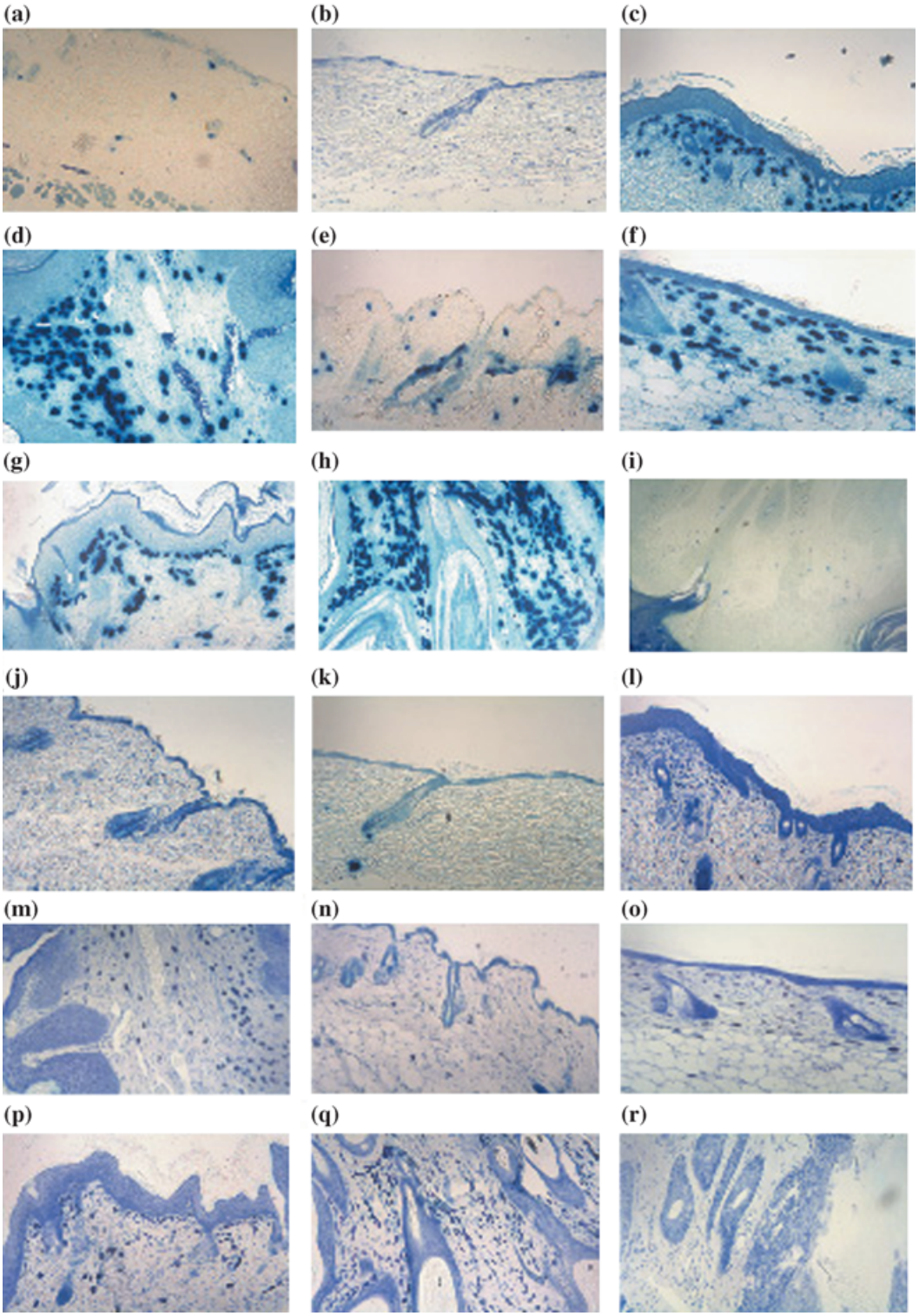

Fig. 2. Distribution of dermal mast cells during mouse skin carcinogenesis. (a–i) Chloroacetate esterase histochemistry; (j–r) toluidine blue stain. Mast cells in: (a,j) epidermis of untreated skin in a wild-type mouse, (b,k) epidermis of 7,12-dimethylbenz[a]anthracene (DBMA) + 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-treated skin in a wild-type mouse; (c,l) hyperplasia in DMBA + TPA-treated skin in a wild-type mouse; (d,m) papilloma in DMBA + TPA-treated skin in a wild-type mouse; (e,n) epidermis of untreated skin in a rasH2 mouse; (f,o) epidermis of DMBA + TPA-treated skin in a rasH2 mouse; (g,p) hyperplasia in DMBA + TPA-treated skin in a rasH2 mouse; (h,q) papilloma in DMBA + TPA-treated skin in a rasH2 mouse; (i,r) absence of mast cells in papilloma in DMBA + TPA-treated skin in a rasH2 *KIT*$^{W/Wv}$ mouse (×200).

the number of mast cells that accumulated in the dermis of treated skin was significantly higher in rasH2 mice than in their wild-type littermates at every stage of chemical skin carcinogenesis, from normal preinitiation skin (53.5 ± 22.2 versus 25.0 ± 7.7, $P = 0.0465$), normal skin 25 weeks after treatment with DMBA + TPA (220.2 ± 127.9 versus 30.2 ± 18.7, $P = 0.0124$), in hyperplasia (333.8 ± 48.6 versus 150.2 ± 69.7, $P = 0.0215$) and in papilloma (546.4 ± 108.4 versus 320.4 ± 118.7, $P = 0.0300$). These data

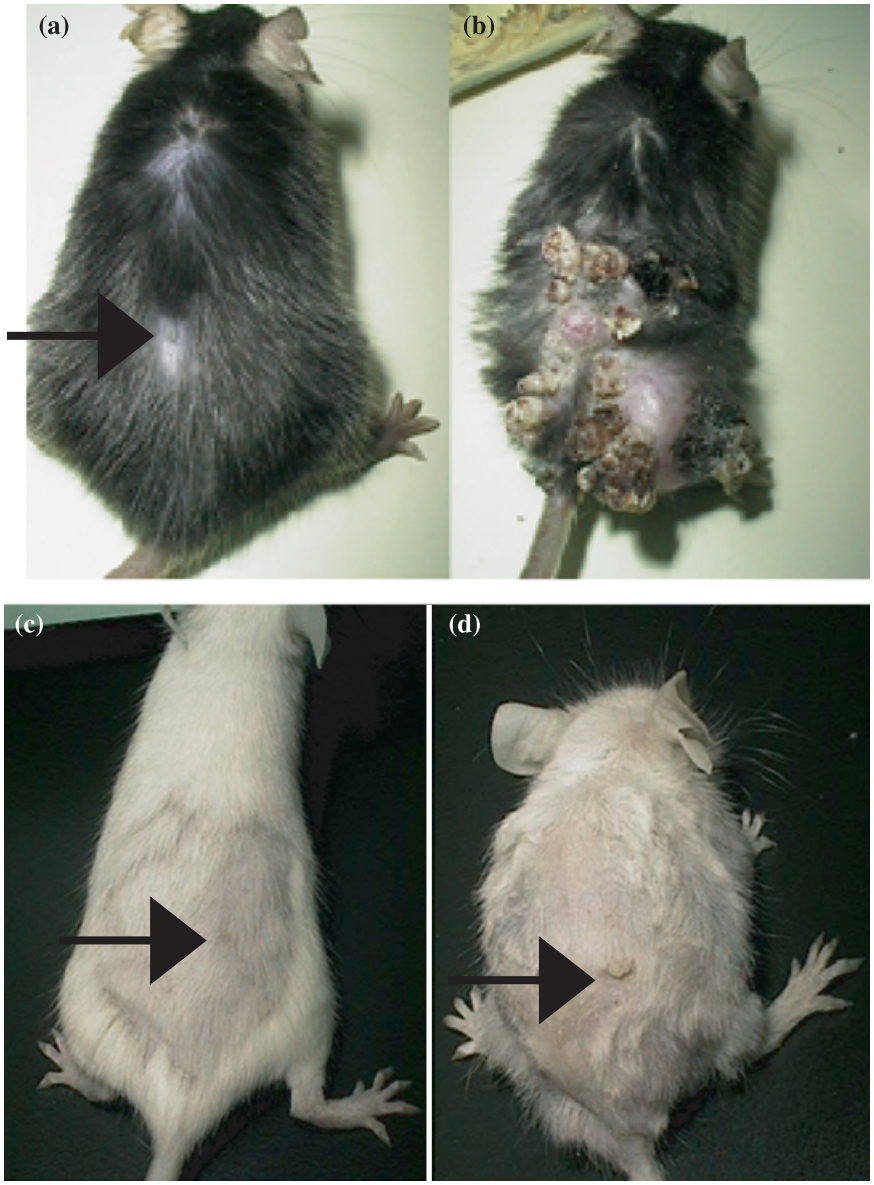

Fig. 3. Gross appearance of skin papillomas. Arrows indicate papillomas. (a) Wild type; (b) rasH2 *KIT*$^{+/+}$; (c) *KIT*$^{W/Wv}$; (d) rasH2 *KIT*$^{W/Wv}$.

indicate that the rasH2 mice accumulated mast cells in the dermis constitutively. The number of mast cells further increased after treatment with DMBA and TPA, and was greater in rasH2 mice than in their wild-type littermates.

Enhanced chemical skin carcinogenesis in rasH2 mice was suppressed by introduction of the *KIT*$^{W/Wv}$ allele. We previously reported that rasH2 mice have enhanced DMBA- and TPA-induced chemical skin carcinogenesis. In rasH2 mice, the latency of tumor formation was shorter than that in their wild-type littermates. Both the number and volume of skin tumors were increased in rasH2 relative to wild-type mice. To determine whether enhanced skin tumor development in rasH2 mice was dependent on the recruitment of epidermal mast cells, we generated rasH2 *KIT*$^{W/Wv}$ mice by crossing rasH2 mice and W or W^{v} *KIT* mutants. In rasH2 *KIT*$^{W/Wv}$ mice, mast cells were not found in the dermis either before or after treatment with DMBA and TPA (Fig. 2). Figure 3 shows the gross appearance of chemical skin papillomas in both rasH2 *KIT*$^{+/+}$ and rasH2 *KIT*$^{W/Wv}$ mice. Papilloma multiplicity was up to 4.6-fold higher in rasH2 *KIT*$^{+/+}$ mice compared with their rasH2 *KIT*$^{W/Wv}$ siblings. At 20 weeks after DMBA treatment, the multiplicity of papillomas in rasH2 *KIT*$^{+/+}$ mice was 27.6

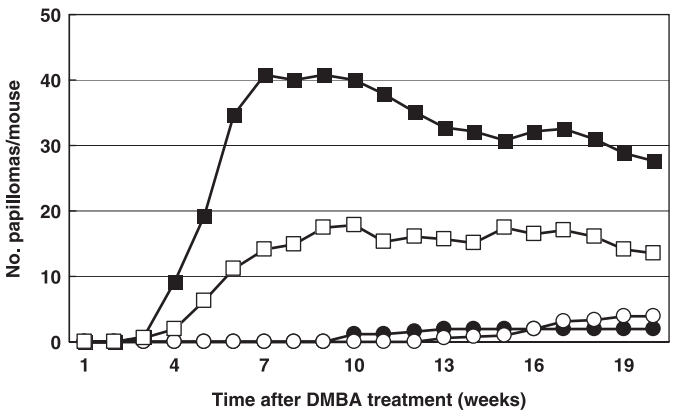

Fig. 4. Average number of papillomas per mouse. (■), rasH2 *KIT*$^{+/+}$; (□), rasH2 *KIT*$^{W/Wv}$; (●) wild-type; (○) *KIT*$^{W/Wv}$.

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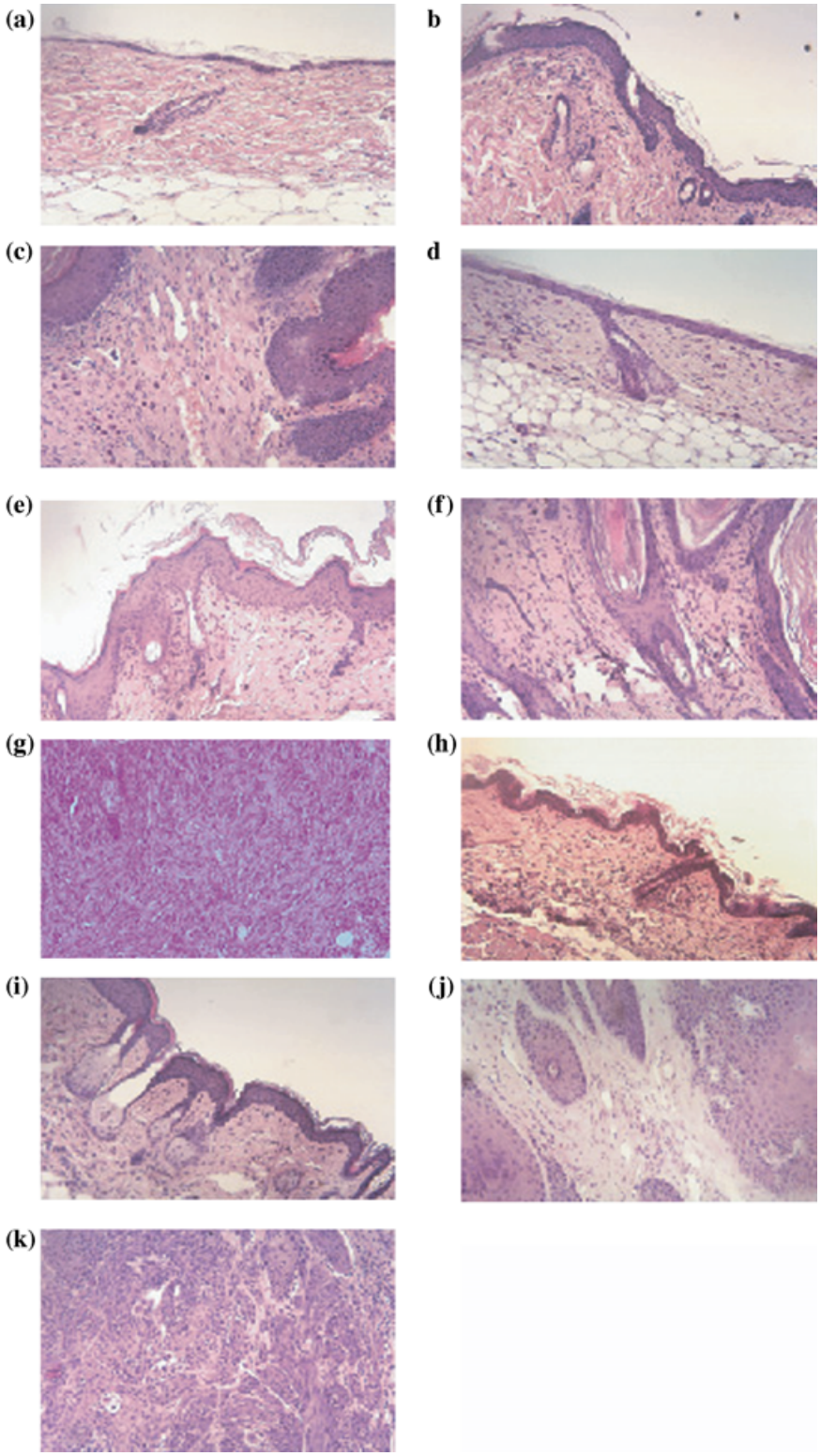

Fig. 5. Histopathological appearance of skin tumors. (a–c) Wild type; (d–g) rasH2 $KIT^{+/+}$; (h–k) rasH2 $KIT^{W/Wv}$. (a,d,h) Epidermis of untreated skin; (b,e,i) hyperplasia in 7,12-dimethylbenz[a]anthracene (DBMA) + 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-treated skin; (c,f,j) papilloma in DMBA + TPA-treated skin; (g,k) carcinoma in DMBA + TPA-treated skin (hematozylin–eosin; ×200).

± 10.4 papillomas/mouse, in contrast to 13.6 ± 9.2 papillomas/mouse in rasH2 $KIT^{W/Wv}$ mice ($P = 0.0334$). Tumor appearance was not delayed in rasH2 $KIT^{W/Wv}$ compared with rasH2 $KIT^{+/+}$ mice (Fig. 4). The volume of tumors (mm^3) was significantly smaller in rasH2 $KIT^{W/Wv}$ than in rasH2 $KIT^{+/+}$ mice (6 weeks after DMBA treatment: 2.8 ± 2.2 vs 42.0 ± 66.6, $P = 0.0032$; 9 weeks: 11.5 ± 14.4 vs 157.9 ± 606.0, $P = 0.0002$; 12 weeks: 29.2 ± 19.9 vs 179.6 ± 726.6, $P = 0.0153$). However, there was no difference in the latency or multiplicity of papillomas between mice without the rasH2 transgene, $KIT^{W/Wv}$ mice and their wild-type littermates (Fig. 4).

Figure 5 shows the histopathological appearance of chemical skin tumors. Upon treatment with DMBA and TPA, cyclin D1 expression is known to be upregulated in papillomas and even in follicular hyperplasias of wild-type mice but not in TPA-treated uninvolved skin.[29] Expression of cyclin D1 was examined using immunohistochemistry. Cyclin D1 was already expressed

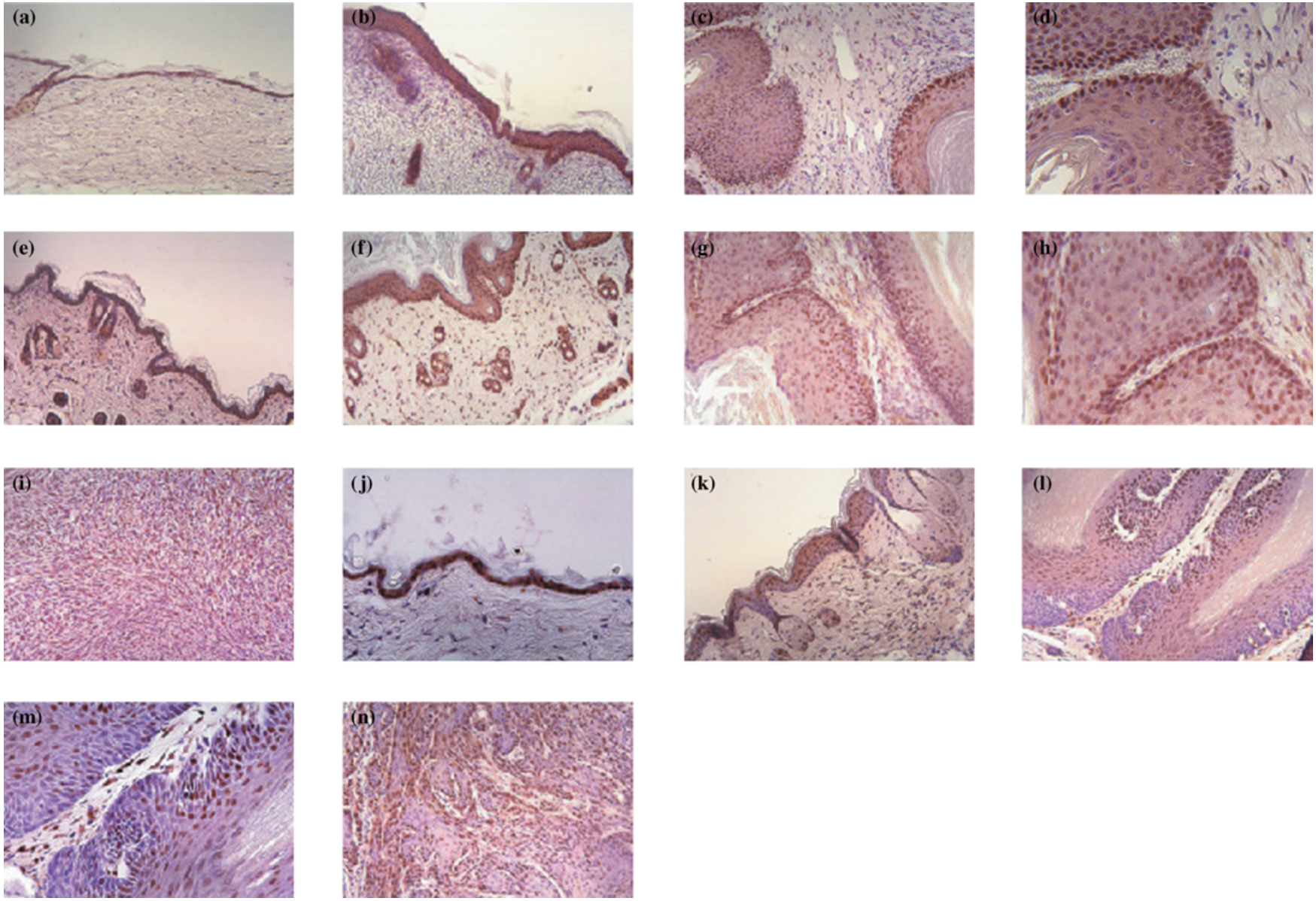

Fig. 6. Expression of cyclin D1 as determined by immunohistochemistry. (a–d) Wild type; (e–i) rasH2 *KIT*$^{+/+}$; (j–n) rasH2 *KIT*$^{W/Wv}$. Expression of cyclin D1 in: (a,e,j) epidermis of untreated skin (×200); (b,f,k) hyperplasia in 7,12-dimethylbenz[a]anthracene (DBMA) + 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-treated skin (×200); (c,g,l) papilloma in DMBA + TPA-treated skin (×200); (d,h,m) papilloma of DMBA + TPA-treated skin (×400); (i,n) carcinoma in DMBA + TPA-treated skin (×200).

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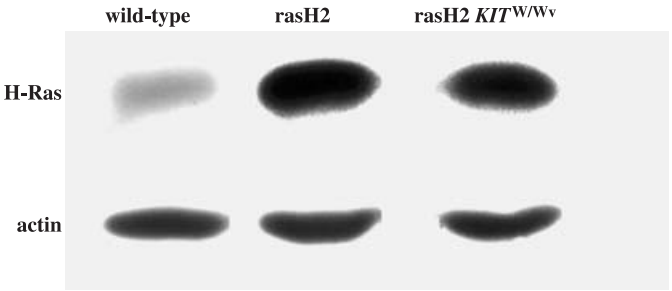

Fig. 7. Western blot analysis of expression of H-RAS protein. Expression of H-RAS protein was higher in rasH2 $KIT^{+/+}$ and rasH2 $KIT^{W/Wv}$ mice than in wild-type mice, but equivalent in rasH2 $KIT^{+/+}$ and rasH2 $KIT^{W/Wv}$ mice.

in the basal keratinocytes of untreated skin in both rasH2 $KIT^{+/+}$ and rasH2 $KIT^{W/Wv}$ mice. In the hyperplasia, papillomas and carcinomas that developed after treatment with DMBA and TPA, expression of cyclin D1 was equivalent in the basal keratinocytes of wild-type, rasH2 $KIT^{+/+}$ and rasH2 $KIT^{W/Wv}$ mice (Fig. 6).

Expression of H-RAS protein in transgenic mice. We examined the expression of H-RAS protein in the back skin of rasH2 $KIT^{+/+}$ and rasH2 $KIT^{W/Wv}$ mice and their wild-type littermates. Western blot analysis showed that expression of H-RAS protein was higher in rasH2 $KIT^{+/+}$ and rasH2 $KIT^{W/Wv}$ mice than in wild-type mice, but equivalent in rasH2 $KIT^{+/+}$ and rasH2 $KIT^{W/Wv}$ mice (Fig. 7).

Discussion

In chemical carcinogenesis, the process of skin tumor development can be subdivided into three stages: initiation, promotion and progression. Initiation is typically induced by the topical application of the carcinogen DMBA. DMBA treatment causes mutations within Ha-*ras* in epidermal cells. These mutations, however, are not sufficient to induce *de novo* transformation. Promotion of tumorigenesis is effected by the topical application of phorbol esters such as TPA to the skin, leading to epithelial cell proliferation and inviting inflammation. Accumulated epidemiological studies support the idea that chronic inflammatory diseases are frequently associated with increased risk of cancers.[30–32] It has been realized that the development of cancers from inflammation might be a process driven by inflammatory cells as well as a variety of mediators, including cytokines, chemokines and enzymes, which together establish an inflammatory microenvironment.[32]

Current evidence suggests that mast cells contribute to tumorigenesis through several mechanisms. Much evidence indicates that mast cells likely induce tumor progression by providing mitogenic stimulation or inducing angiogenesis.[33–38] Data from experimental studies support the hypothesis that neovascularization is induced by mast cell-derived angiogenic mediators or growth factors.[14] Mast cells release several mitogenic mediators, including fibroblast growth factor-2 and interleukin-8,[39] and mast cell proteases reorganize the stroma to facilitate endothelial cell migration. Via the action of their own proteases, and indirectly via interactions with other cells, mast cells participate in degradation of the matrix, which is a process required for tumor spread. In several malignancies, mast cell density has been found to correlate with increased risk of metastasis and poor prognosis.[40–43] These studies emphasize the role of mast cells in accelerating the progression of cancers by providing scaffolds in the pro-progressive microenvironment. In a recent study it was found that allograft tolerance cannot be induced in mast-cell-deficient mice, showing that mast cells are crucial for allograft tolerance. Thus, in addition to secreting various cytokines that induce angiogenesis and remodeling stroma, mast cells may play a role in the immune tolerance of tumor cells.[44]

In the present study, we showed that the introduction of $KIT^{W/Wv}$ suppresses H-ras-induced skin carcinogenesis in rasH2. Contrary to our findings, Tanooka *et al*. reported that mast cell-deficient $KIT^{W/Wv}$ mice had an increased tumor incidence after subcutaneous treatment with 3-methylcholanthrene (MCA) compared with that in normal congenic mice treated in the same way.[45] Their paper is indeed a landmark paper that recognizes the involvement of mast cells in carcinogenesis in the microenvironment. Their study showed that deletion of mast cells in $KIT^{W/Wv}$ mice stimulated early carcinogenesis without changing the latency of tumor formation. The effect of mast cell depletion was restored by the reconstitution of bone marrow transplant. However, the experimental conditions are different between the two studies. First, different carcinogens could activate disparate signaling pathways in chemical carcinogenesis. Second, the cell types of developed tumors were different between their study and ours. Their study and others show that skin carcinogenesis induced by DMBA + TPA primarily develops papillomas, and then carcinomas develop subsequently among papilomas,[7–9] meanwhile MCA develops mainly sarcomas. A recent study using a conditional knockout of p53 shows that restoration of p53 expression after the deletion of p53 induces apoptosis in developed lymphoma, but does not induce apoptosis in sarcoma.[46] The involvement of immune-responsible cells can work differently in different tumor cell types, even in the same genetic background. Thus, it may not be contradictory to see the different role of mast cells in sarcoma induced by MCA and papilloma by DMBA + TPA. Third, their study saw a difference in the number of mice that gave rise to tumors, but our study saw a difference in the latency and number of tumors in individual mice. Thus, it is likely that immune-responsible cells located at the interface of tumor and normal mesenchyme can act to both stimulate and inhibit carcinogenesis. However, the role of mast cells in the present study lacks robust evidence as proved by the study of bone marrow reconstitution.

Moreover, in chronic inflammation, mast cells are not unique among inflammatory cells in potentiation of neoplastic processes. Polymorphonuclear cells (PMN), macrophages and activated T lymphocytes can also contribute to malignancies by releasing proteases, angiogenic factors and chemokines.[47,48] Wershil *et al*. reported that the inflammatory response *per se* is relatively weak in the skin of $KIT^{W/Wv}$ mice compared with wild type upon treatment with PMA.[28] Introduction of W/W^v *KIT* alleles might have changed the immune response to PMA in addition to the deficiency of mast cells.

Many mutations have been described for the dominant-white spotting (*W*) locus on mouse chromosome 5, which encodes the c-kit receptor. These mutations are known to induce pleiotropic effects, such as sterility, macrocytic anemia and depletion of melanocytes and mast cells.[49] In W/W^v double heterozygous mutant transgenic mice, c-kit activity is decreased to less than 10% of the levels in wild-type mice.[50] In addition to causing mast cell deficiency, impaired c-kit kinase in mutant mice could interfere with skin carcinogenesis via several signal transduction pathways, given that numerous Kit interactions lead to activation of multiple signal transduction pathways, including those mediated by mitogen-activated protein kinase[51] and phosphatidylinositol 3-kinase.[44] In the current study, because there was no difference in the tumor latency and the number of papillomas between wild-type and W/W^v mice, we infer that impairment of c-kit kinase in W/W^v mice did not significantly affect chemical skin carcinogenesis *per se*. Biochemical and genetic evidence shows that cyclin D1 is a critical target for oncogenic *ras* in mouse skin. *Ras*-mediated skin tumorigenesis is substantially reduced in a cyclin D1-deficient background.[29] Indeed, in rasH2 $KIT^{W/Wv}$ mice, expression of cyclin D1 in the keratinocytes was equivalent to that in rasH2 $KIT^{+/+}$ mice. Thus, expression of cyclin D1 was not dependent on the presence of accumulated

mast cells. Impairment of c-kit kinase in the W/W^v mutant did not affect the expression of cyclin D1 via activation of the ras signaling cascade in rasH2 mice. These data indicate that the deficiency of mast cells interfered with carcinogenesis at downstream points in cascades mediated by the expression of cyclin D1.

In conclusion, the current study was the first, to our knowledge, to show that inhibition of c-kit decreases H-ras-induced skin carcinogenesis. The suppression of c-kit may be a unique and effective target as a preclinical model of cancer treatment where the activation of H-ras has a significant role. Targeting mast cells could also be a potential strategy for treating malignancies that are dependent on the activation of H-ras.

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