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Letter to the Editor

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Widespread spontaneous hyperproliferation, melanosis and melanoma in *Hgf-Cdk4^{R24C}* mice

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Seeking a model of spontaneous metastatic melanoma, we imported the *Hgf-Cdk4^{R24C}* mouse line in which overexpression of hepatocyte growth factor (*Hgf*) promotes melanocyte proliferation and migration and the clinically relevant mutation in cyclin-dependent kinase 4 (*Cdk4^{R24C}*) prevents binding to the tumor suppressor protein p16 [1]. These mice have been reported to spontaneously develop a spectrum of primary melanomas, with 61% showing progressively growing melanomas at 1 year of age (74% nodular melanomas, 26% flat melanomas) [2]. The tumours appear mostly on the back (80%) and more rarely in other locations such as the ear, nose, belly, extremities, anus and tail (2–5%/site), with metastasis into the draining lymph nodes in all mice [2]. More recently, a study of the eyes of 11-month-old *Hgf-Cdk4^{R24C}* mice found altered corneal stromal morphology, but no evidence of spontaneous ocular melanoma [3].

In our facility, *Hgf-Cdk4^{R24C/R24C}* mice were generated by intercrossing *Hgf-Cdk4^{R24C/R24C}* male mice with *Cdk4^{R24C/R24C}* female mice (C57BL/6 background; the use and housing conditions of mice are detailed in Supplementary Data Table 1 (Supplemental digital content 1, <http://links.lww.com/MR/A20>)). Females were predominantly used in this study as litters from this mouse line were typically small and mice carrying the *Hgf* allele had sub-Mendelian ratios. Thus, to

avoid having singly housed males (in the case of only one male from a litter carrying the *Hgf* allele), we mostly kept females for tumour watch studies as they could be group housed irrespective of their age. *Hgf-Cdk4^{R24C/R24C}* mice (12 females, 3 males) were left to age with daily visual inspections, and typically were killed because of hyperproliferation of their anogenital areas at 341 ± 61 days of age (range: 178–429 days). At the time of necropsy, we observed a range of tissue sites undergoing hyperproliferation, with the presence of melanosis and the development of melanoma (and other tumours) in many cases (Table 1). All mice showed mixed anal mucosal melanoma/perianal skin malignant melanoma (Fig. 1a–c) with concomitant vulval/penile mucosal melanoma (Fig. 1d and e). Both flat and papillary skin melanomas (Fig. 1f) were noted in all mice, with a few cases of nodular melanoma (Fig. 1g). A high incidence of mucosal melanoma of the lips was also observed, as well as two cases of mucosal melanoma of the snout. Opacity and ulcerations of the eyes and/or discharge from the eyes were a recurrent observation; all eyes sampled showed retinal melanosis (Fig. 1h) with increased melanin pigmentation of the retinal pigment epithelium (and usually the iris/uveal tract), some with a possible increase in numbers of retinal pigment epithelial cells, but no evidence of invasive melanoma of the retina or the uveal tract (with one case showing a concomitant conjunctival melanoma). All hard-erian glands sampled showed hyperplasia and adenoma formation (sometimes >1). All brains sampled showed

Table 1 Tissue distribution and frequency of melanosis and tumour development in *Hgf-Cdk4^{R24C/R24C}* mice

Pathology	Frequency [n/N (%)]
Perianal skin melanoma/anal canal mucosal melanoma	15/15 (100)
Vulval mucosal melanoma	12/12 (100) ^a
Penile melanoma	3/3 (100)
Skin nodular melanoma	3/15 (20)
Skin flat melanoma	15/15 (100)
Skin papillary melanoma	15/15 (100)
Lip mucosal melanoma	12/15 (80)
Snout mucosal melanoma	2/15 (13)
Eye harderian gland hyperplasia and adenoma	14/14 (100) ^b
Eye retinal melanosis	14/14 (100) ^{b,c}
Brain meningeal melanosis	14/14 (100) ^b
Liver hepatocellular carcinoma	2/15 (13)
Lymph node metastatic melanoma	10/15 (66) ^d

Cdk4, cyclin-dependent kinase 4; *Hgf*, hepatocyte growth factor.

^aOne mouse also developed a uterine/cervical melanoma.

^bFor one mouse, the eyes, harderian gland and brain were not collected for analysis.

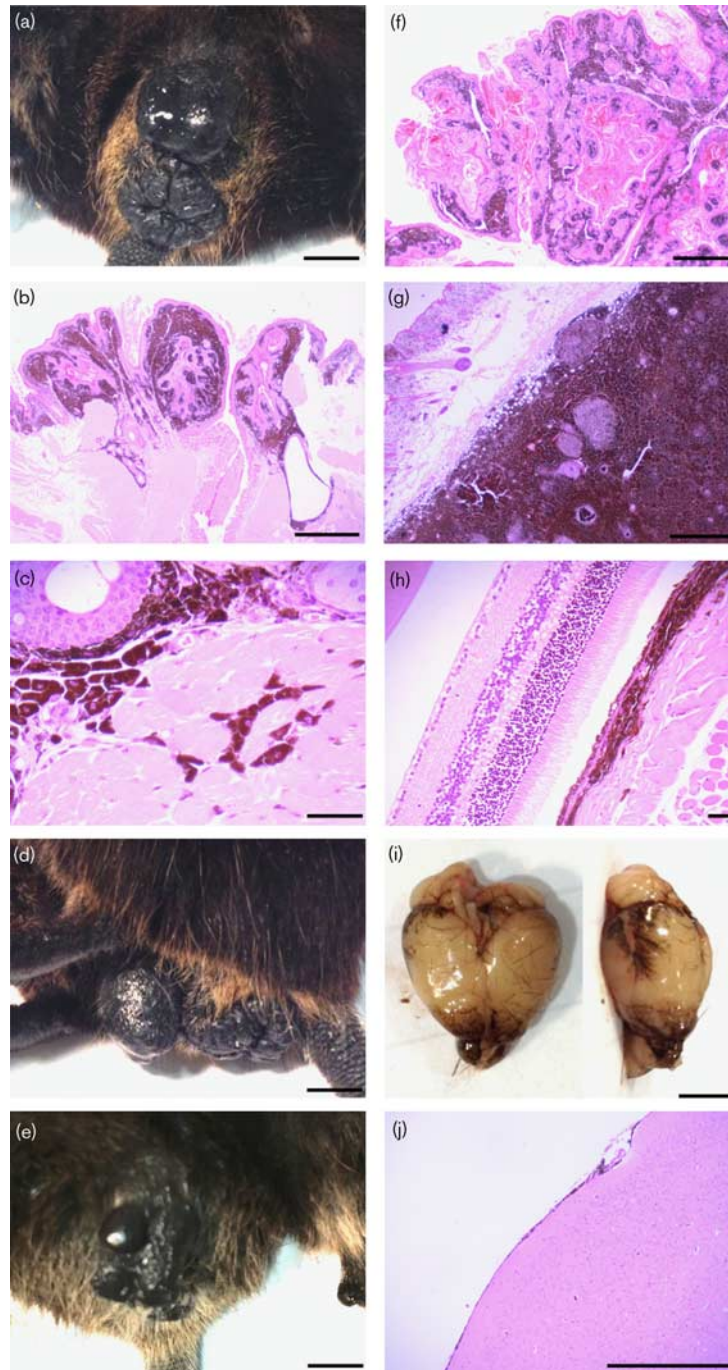
^cOne mouse also developed conjunctival melanoma.

^d5/15 (33%) mice showed the presence of pigmented macrophages in lymph nodes, but no definite melanoma.

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Fig. 1



Macroscopic and histological images of tissue melanosis and melanoma in *Hgf-Cdk4^{R24C}* mice. (a) Macroscopic image of a perianal (and vulval) melanoma (the scale bar is 0.4 cm). (b, c) Haematoxylin and eosin (H&E)-stained section of a perianal melanoma, showing a papillary melanoma with both junctional and dermal melanoma components with the melanoma extending up around the anal canal close to the anorectal junction (the scale bar is 1 mm, b), and showing invasion of atypical, malignant, heavily pigmented melanoma cells into muscle (the scale bar is 50 μ m, c). (d) Macroscopic image of a vulval (and anal) melanoma (the scale bar is 0.4 cm). (e) Macroscopic image of a penile melanoma (the scale bar is 0.4 cm). (f) H&E-stained section of a skin papillary melanoma, showing junctional and dermal melanomatous components admixed with squamous epithelium and keratinous material showing a papillomatous architecture (the scale bar is 1 mm). (g) H&E-stained section of skin showing nodular melanoma in the subepithelial tissue, mostly heavily pigmented with a few small oval foci of loss of pigmentation (the scale bar is 1 mm). (h) H&E-stained section of retinal melanosis, showing a thickened and prominent pigmented layer under the retina because of overactivity of the retinal pigment epithelium with pigment extending into the underlying muscle and other tissue (the scale bar is 50 μ m). (i) Macroscopic image of a brain showing meningeal melanosis (the scale bar is 0.4 cm). (j) H&E-stained section of meningeal melanosis, showing variably increased pigment within the thin meningeal layer on the surface of the brain (the scale bar is 1 mm). All images are representative of those observed across the cohort (as detailed in Table 1). *Cdk4*, cyclin-dependent kinase 4; *Hgf*, hepatocyte growth factor.

meningeal melanosis with increased melanin pigmentation of the meninges, some with a probable increase in numbers of meningeal melanocytes, but no evidence of invasive melanoma infiltrating brain tissue (Fig. 1i and j). A few livers showed hepatocellular carcinoma and most livers showed increased melanin pigment. Metastasis to the lymph nodes was noted in 10/15 mice, with the remaining 5/15 mice showing the presence of pigmented macrophages in the lymph nodes.

Thus, in our facility, we had an increased incidence of mucosal melanoma, retinal melanosis and brain meningeal melanosis in preference to cutaneous melanoma, which is in contrast to previous reports [2], although spontaneous melanocytic hyperplasia of the mucosa, particularly of the perianal and genital mucosa, has been observed previously, but not reported (Thomas Tuting, unpublished observations). Also, the cutaneous melanomas that we observed were of the flat and/or papillary types, less commonly the nodular type, which has been reported previously to be the predominant one. Finally, we did not find any alterations in pigmentation or cellular components of the ocular epithelium or the posterior segment that have been reported previously [3]. Essentially, the oncogenic mutation in this model appears to be capable of driving almost all melanocytes to hyperproliferation and subsequent neoplasia, with more neoplasia in some sites than others depending on whether additional mutational events are required. The reason for a shift in phenotype between the animal facilities is unclear and further investigation is undoubtedly warranted. Although these mice were maintained on a C57BL/6J background, we cannot account for genetic drift of the C57BL/6J colonies between the different institutes, which could influence tumour growth [4]. It is tempting to speculate that environmental factors such as differences in the housing

conditions of the mice between the two facilities (detailed in Supplementary Data Table 1, Supplemental digital content 1, <http://links.lww.com/MR/A20>) may be playing a role as these factors have been shown to affect tumour growth [4]; indeed, the presence of harderian gland hyperplasia and adenoma formation has been observed in aged mice from other lines in our facility. Similarly, the microbiota of the mice, which would undoubtedly differ between the two facilities, affect many aspects of physiology, most importantly tumour-immune cell interactions, and thus can play a major role in regulating the initiation, progression and dissemination of cancer [5].

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Conflicts of interest

There are no conflicts of interest.

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