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Squamous Cell Carcinomas of the Skin at Ear Tag Sites in Aged FVB/N Mice

Beverly W. Baron, MD,[1,*] George Langan, DVM,[2] Dezheng Huo, PhD,[3] Joseph M. Baron, MD,[4] and Anthony Montag, MD[1]

We report the development of squamous cell carcinomas (SCCs) of the skin at or near the site of ear tags composed of a nickel–copper alloy and used for identification during the course of a long-term study of incipient congenic FVB/N mice containing the human *BCL6* transgene (FVB.Cg-Tg[tetO-BCL6]Bbn Tg[EμSR-tTa]83Bop), their littermate controls, and wild-type FVB/N. Of a total population of 160 mice, 14 (8.8%) developed SCCs in the tagged (right) ear after a median observation period of 25 months, but none of the animals developed tumors in the opposite ear (*P* = 0.0001). Nine of the fourteen mice with SCCs had to be euthanized because they were thought to be in distress from the ear condition, but the remaining five died or were euthanized for other reasons related to the research study. These animals ranged in age from 331 to 921 days at the time of death. Five of the tumors were well-differentiated (grade 1) SCCs; the remainder were grade 3 and tended to be deeply invasive neoplasms with undifferentiated areas containing a spindle cell component. One of these metastasized to kidney. When using the FVB/N mouse strain for long-term studies, it is necessary to consider that nearly 9% of the population may develop SCCs at or near ear-tag sites that may necessitate early removal of the animal.

Various malignancies have been reported to develop in association with prostheses, bone plates, and bone pins in humans and other mammals. These usually have been soft tissue sarcomas, but they also have included hemangioendotheliomas, histiocytomas, Ewing's sarcomas, and osteosarcomas (21). Waalkes and colleagues (21) reported inflammatory and neoplastic lesions at the site of metallic identification tags in Wistar rats. These investigators used tags similar to the ones we used, which consisted of a nickel–copper alloy. Their animals developed a variety of benign and malignant conditions at the ear tag sites, but these did not include squamous cell carcinomas (SCCs). In one of their studies, 8.3% of rats developed tumors, most commonly osteosarcomas. The only other report we could find noting a condition associated with ear tagging described a white-tailed deer (*Odocoileus virginianus*) with debilitating ossifying fibromas near aluminum tags (18). In humans, there has been one report of SCC developing in association with a mandibular staple bone plate (9), and an additional report of squamous cell papillomatosis of the esophagus after placement of a metal stent containing a number of metals, including nickel (12). Waalkes and colleagues (21) hypothesized that enhanced metal ion release from the tag because of local acidic pH due to inflammatory reaction to the tag plays a key role in tumor incidence in their rats.

We report an overall incidence of 8.8% SCCs in the ear in which a metallic identification tag was placed in incipient congenic FVB/N mice (FVB.Cg-Tg[tetO-BCL6]Bbn Tg[EμSR-tTa]83Bop and their littermate controls) as well as wild-type FVB/N that were observed long-term during the course of another study (2). The development of this kind of neoplasm has not been reported previously in mice in association with ear tags. Interestingly, administration of *N*-ethyl-*N*-nitrosourea (ENU), a carcinogen that is known to induce DNA mutations, did not enhance the incidence of SCCs associated with ear tags.

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Departments of [1]Pathology, [2]Surgery, [3]Health Studies, and [4]Medicine, The University of Chicago, Chicago, Illinois 60637.
[*]Corresponding author.

Materials and Methods

Animals. All animal procedures were performed in accordance with the humane care and use standards approved by the Institutional Care and Use Committee at The University of Chicago. The University of Chicago is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. The findings reported here are from observations made during the course of another study (2). In an effort to develop a mouse lymphoma model, CD-1 mice containing the human *BCL6* transgene (tet-*o*-BCL6) were rederived through embryo transfer (because of a 1999 outbreak of mouse hepatitis virus) in FVB/Ntac mice (Taconic Farms, Germantown, N.Y.). These animals were crossed to transgenic FVB/N mice expressing the tetracycline-transactivating protein (tTA) under control of the immunoglobulin enhancer and the SRα promoter (Tg[EμSR-tTa]83Bop) obtained from Felsher and Bishop (University of California, San Francisco; 7) in order to express the *BCL6* transgene specifically in lymphocytes. For additional backcrosses, wild-type FVB/NCr mice (weanlings to young adults) were purchased from the National Cancer Institute (Frederick, Md.).

The resulting incipient congenic FVB/N mice (FVB.Cg-Tg[tetO-BCL6]Bbn Tg[EμSR-tTa]83Bop), their littermate controls, and wild-type FVB/NCr were placed in individually ventilated cages containing corncob bedding in a room on a 12:12-h light:dark cycle and were fed a standard irradiated rodent chow and provided acidified water ad libitum. They were housed one to five animals per cage in specific pathogen-free facilities. Specific pathogen-free status was maintained via testing of sentinels exposed to dirty bedding from experimental cages. The mice were monitored for and remained free of the following pathogens: mouse hepatitis virus, mouse minute virus, mouse rotavirus, mouse

encephalomyelitis virus, lymphocytic choriomeningitis virus, mouse parvovirus, pneumonia virus of mice, Sendai virus, mouse cytomegalovirus, Hantaan virus, reovirus 3, ectromelia virus, mouse polyoma virus, mouse adenovirus, *Mycoplasma pulmonis*, *Salmonella* spp., *Citrobacter rodentium, Clostridium piliforme*, cilia-associated respiratory bacillus, endoparasties, and ectoparasites.

On arrival in our facilities and at weaning (3 to 4 weeks of age), a metallic identification tag recommended by our animal center was placed by experienced animal care staff through the skin and cartilage at the base of the pinna of the right ear by using a specifically designed applicator. The tags consisted of a nickel–copper alloy containing 63 to 70% nickel, 0.3% (maximum) carbon, 2% (maximum) magnesium, 2.5% iron, 0.024% (maximum) sulfur, and 0.5% (maximum) silicon, with the balance being copper (~30%). The mice were divided into six groups and observed during a chronic study of lymphoma (2). Mice that developed incurable conditions were euthanized by carbon dioxide inhalation.

For the previously described study (2), a subgroup of mice transgenic for *BCL6* (FVB.Cg-Tg[tetO-BCL6]Bbn Tg[EμSR-tTa]83Bop) backcrossed five times to FVB/N and their littermate controls was injected once intraperitoneally with ENU (Sigma, St. Louis, Mo.; 100 mg/kg dissolved in phosphate buffered saline [PBS], pH 6) or with PBS only. Groups 1 (transgenics) and 3 (controls) were injected with ENU, whereas Groups 2 (transgenics) and 4 (controls) received PBS. Two wild-type FVB/NCr mice were included (one each in Groups 3 and 4). Littermate controls received injections at the same ages (61 to 297 days of age) as did the transgenic mice. Each mouse that died was necropsied. In addition, a cohort of noninjected aged mice (Group 5, transgenics; Group 6, controls) backcrossed four times to FVB/N was followed until euthanasia at the end of the chronic study (2). One wild-type FVB/NCr mouse was included in Group 6.

Histology. Routine necropsies included gross and microscopic examination of representative sections of all tumors noted, in addition to lungs, liver, kidneys, thymus, spleen, and intestines. Other organs (e.g., bone marrow) were included when pertinent. Tissues were fixed in 10% buffered formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. All noteworthy lesions and tumors were evaluated by two pathologists certified by the American Board of Pathology, one of whom was blinded as to mouse line and treatment. Both pathologists had prior experience in evaluation of mouse tissues. SCCs were defined as grade 1 if they were well-differentiated neoplasms with obvious cornification and horn pearl formation, grade 2 if they were less differentiated but if 5 to 50% of the tumor showed cornification, and grade 3 if less than 5% of the tumor was well differentiated and had areas showing a spindle cell component and deep invasion into the underlying tissue. All of the tumors could be identified as SCCs from foci of recognizable squamous cells and, usually, horn pearl formation.

Statistical analysis. Statistical analyses were done with Stata 8.2 (StataCorp, College Station, Tex.). Because ear tumors did not occur in animals younger than 300 days, we analyzed mice 300 days old or older. One transgenic mouse injected with PBS had to be excluded because of severe autolysis after death. The total analyzable population consisted of 160 mice, with balanced numbers of female (86 [54%]) and male (74 [46%]) mice. A sign test was used to examine whether ear tumors were more likely to occur at the site of tagging. The incidence of ear skin cancer between the groups or gender was compared by use of the log-rank test.

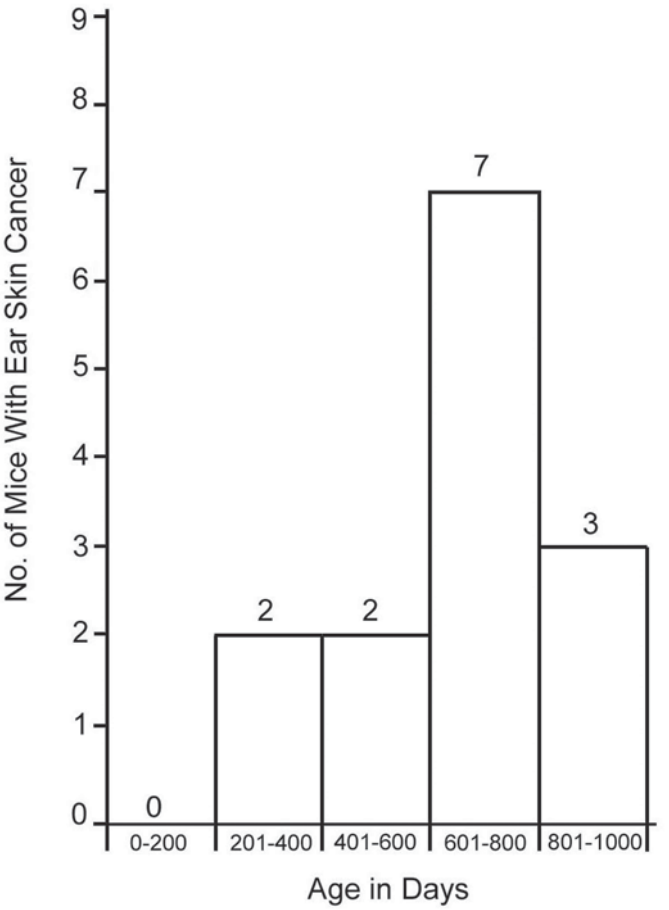

Figure 1. The number of mice that developed SCCs at ear tag sites during the course of observation at each 200-day interval.

Results

Squamous cell carcinomas at ear tag sites. At the time of analysis, 146 (91.3%) of the 160 total analyzable mice had died, and 14 of the 160 mice (8.8%) had developed SCC in the tagged (right) ear, but no mouse developed SCC in the left ear ($P = 0.0001$). Of the SCCs, one occurred among the 21 ENU-injected transgenic animals (Group 1; all dead), one among the 22 PBS-injected transgenic mice (Group 2; 12 still living), two among the 26 ENU-injected controls (Group 3; all dead), five among the 27 PBS-injected controls (Group 4; two still living), two among the 25 noninjected transgenics (Group 5; all dead), and three among the 39 noninjected controls (Group 6; all dead). There were no statistically significant differences in incidence among these groups ($P = 0.52$). Four of these cancers occurred in the 74 male mice and ten in the 86 female mice ($P = 0.19$). Nine of the mice with SCCs had to be euthanized because they were thought to be in distress from the ear condition, but five of the tumors occurred in mice that died from other conditions or were euthanized for unrelated reasons. At death, the 14 mice with SCCs of ear skin ranged in age from 331 to 921 days, with a median age at death of 693 days (Fig. 1). The mouse with the earliest appearance of

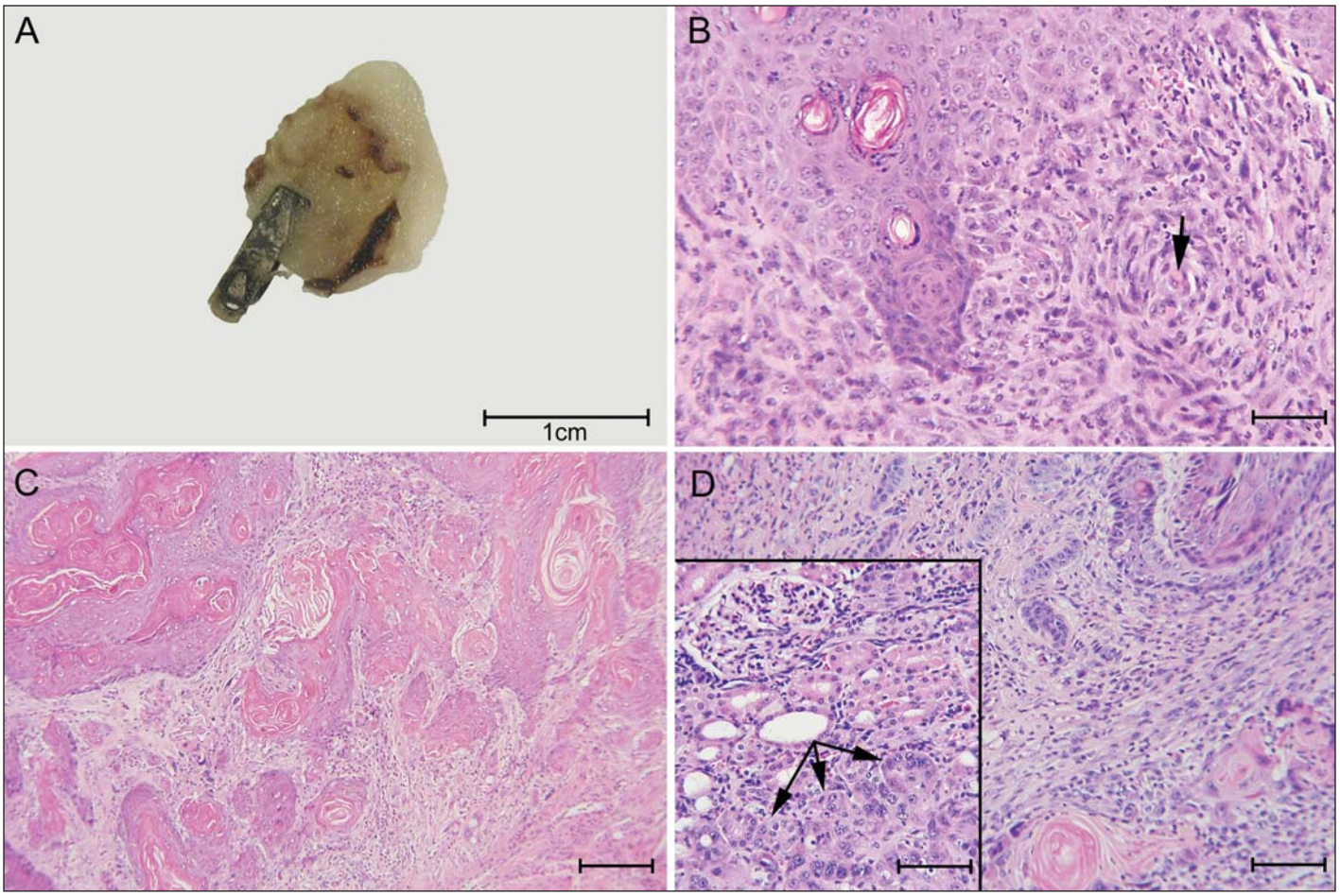

Figure 2. SCCs at ear tag sites. (A) Formalin-fixed right ear from mouse showing the metallic tag surrounded by a tumor mass. (B) Photomicrograph of hematoxylin and eosin-stained histologic section of tumor in (A) reveals a grade 3 SCC arising from the squamous layer of the epidermis and infiltrating the dermis. Individual cell keratinization is present (arrow) in addition to an extensive spindle cell component. Bar, 60 µm (C) Example of grade 1 (well-differentiated) SCC of the skin, showing extensive horn pearl formation. Bar, 40 µm (D) Grade 3 SCC containing a spindle cell component in addition to horn pearls (lower right). This tumor metastasized to kidney (inset). Bar, 48 µm. The inset depicts a glomerulus (upper left) and the metastatic SCC (lower right, arrows). Bar, 60 µm.

ear skin SCC was the wild-type FVB/NCr in Group 6. Usually the mice had to be euthanized within a few days of discovery of the tumor because of distress from scratching, bleeding, or inflammation at the site.

Grossly the tumors were firm, flesh-colored to brown-red masses that often were ulcerated or encrusted with dried blood. They measured 0.7 to 2 cm in largest diameter, and when a tag was still present, the cancer often encircled it (Fig. 2A). Five of the cancers were well differentiated (grade 1, Fig. 2C), but nine invaded the underlying tissue more deeply and had areas of poorly differentiated spindle cells (grade 3, Fig. 2, B and D), although they still could be identified as being of squamous cell origin by the presence of better differentiated regions with horn pearls (Fig. 2D) or by the detection of keratinization of individual cells (Fig. 2B). One grade 3 tumor metastasized to the lower pole of the left kidney (Fig. 2D). All of the tumors that occurred were SCCs, and none showed histologic evidence of neoplastic transformation from another cell type.

Squamous cell carcinomas at other sites. Nine additional SCCs were noted at other sites—three in the lower esophagus at the gastroesophageal junction (two in Group 1 animals and one in a Group 6 mouse), one in the mouth (Group 4), and five in skin—two in the chest area (Group 1), one overlying the left shoulder (Group 4), one overlying the hip (Group 5), and one overlying the gluteal area (Group 3); this tumor had extensive spindle cell histology. One of the gastroesophageal carcinomas in Group 1 metastasized to lung, liver, pancreas, and pelvis. All of these tumors led to euthanasia because of distress or to demise of the mouse.

Discussion

In the course of another study, we observed the development of SCCs at or near the sites of metallic identification ear tags in 14 mice, which represent 8.8% of the 160 mice observed over a long-term period (300 days or more). Although SCCs were noted also at other sites—mouth, gastroesophageal junction, and other skin areas—none was detected in the opposite ear. The cause of the SCCs at the other sites is not known. A carcinogen, ENU, which induces mutations in DNA, was injected in a subgroup (n = 47) of the mice, but these animals did not show an increase in the incidence of SCC either at the location of the ear tag or in other sites. Similarly, a subgroup of mice (n = 68) transgenic for the human *BCL6* gene, which is known to play a role in lymphomatous transformation, did not demonstrate an increased incidence of SCCs.

Neoplastic lesions in conjunction with tagging of animals have

not been reported frequently. A white-tailed deer developed fibromas near aluminum tags (18), and Waalkes and colleagues noted 14 tumors at sites of metallic identification ear tags in 168 Wistar rats (8.3%) during the last six months of a 2-year study (21). In that study, nine of the tumors were, at least in part, osteosarcomas, but no SCCs were reported. Those metal tags, consisting of 65.3% ± 3% nickel, 32.4% ± 3.7% copper, 1.27% ± 0.36% iron, 0.85% ± 0.14% manganese, and 0.2% ± 0.03% chromium, were very similar in composition to those we used. As Waalkes and colleagues note, if the metals of the tags were important in carcinogenesis, then it would seem that nickel, which has been documented to be carcinogenic, is the most likely etiologic agent, although a role of the other metallic components could not be excluded.

Nickel refinery workers develop both upper and lower respiratory tract cancers; 48% of upper respiratory tract and 67% of the lung cancers that develop after nickel exposure are SCCs. These tumors appear after a long latency period—15 to 24 years after initial exposure for nasal cancers and 3 to 14 years for lung cancers (5). Further, a single intramuscular injection of a nickel compound in Fischer rats led to the appearance of various kinds of sarcomas at the injection site within 2 years (5). Faccione and colleagues (6) found that nickel released from orthodontic appliances could induce DNA damage in oral mucosal cells. Similarly, Cangul and coworkers (4) reported that nickel compounds selectively damage chromosomal material and postulated that gene silencing is an important event in nickel-induced carcinogenesis. Epigenetic effects of nickel include alteration in gene expression due to DNA hypermethylation as well as histone hypoacetylation, and activation or silencing of certain genes and transcription factors, particularly those involved in cellular response to hypoxia (13). In addition, nickel (II) in noncytotoxic quantities can inhibit DNA excision repair (25), a trait that may contribute to the metal's genotoxicity.

It also has been shown that copper-based alloys are susceptible to corrosion and dissolution, rendering them cytotoxic (1). Copper catalyzes reactive oxygen species generation from various organic carcinogens, causing oxidative DNA damage (14). In a study of growth of Scots pine, the presence of copper in soil seemed to increase nickel toxicity (16). In addition, Wong and colleagues (23) noted that the toxic interactive effects between these and a number of other metals may be synergistic.

Some papillomaviruses have been associated with tumorigenic potential (10) in humans and other mammals, although the virus's role in the etiology of nonmelanoma skin cancers, especially in immunocompetent patients, is not clear (17). Forslund and coworkers (8) found a high incidence of cutaneous human papillomavirus DNA on the top of skin tumors as well as on healthy skin, but they identified it less commonly within the skin lesions. Bovine papillomavirus induces papillomas of the cutaneous or mucosal epithelium in cattle, and these papillomas occasionally transform to SCC (3). Papillomaviruses also have been detected in cutaneous fibromas of white-tailed and mule deer (20), and cutaneous fibromas can be transmitted to white-tailed deer by inoculation of crude fibroma extracts (19). Transgenic mice harboring human papillomavirus type 16 E6/E7 driven by human keratin 14 promoter developed epithelial hyperplasia and hyperkeratosis (15). Further, almost 100% of transgenic mice carrying oncogene E6 of the *Mastomys natalensis* papillomavirus (a rodent papillomavirus that causes keratoacanthomas in its natural host *M. natalensis*) developed SCCs as compared with 10% of their nontransgenic littermates under conditions of experimental two-stage skin carcinogenesis (10). Although it seems unlikely that the SCCs in the tagged ears of our mice were caused exclusively by papillomaviruses, a role of such viruses in the development of these lesions cannot be excluded entirely.

FVB/N mice are inbred for homozygosity of the allele determining susceptibility to B-type Friend leukemia virus and are being used widely for establishment of transgenic lines containing active oncogenes (11). These mice appear to be at particular risk for development of SCCs in the skin induced by exposure to certain carcinogens. Hennings and colleagues (11) exposed the skin of several mouse strains to chemical carcinogens, including 7,12-dim ethylbenz[*a*]anthracene (DMBA), DMBA initiation–12-*O*-tetradecanoylphorbol-13-acetate (TPA) promotion, and TPA alone. In that study, 50% of papillomas progressed to SCCs in the FVB/N strain, as compared with 9.15% in SENCAR, 15% in C57CL/6, 23.1% in BALB/c, and 37.5% in CD-1 mice. The first cancers developed after 14 weeks in FVB/N, 24 weeks in SENCAR, 26 weeks in CD-1 and C57BL/6, and 34 weeks in BALB/c mice. Wang and coworkers (22) studied the susceptibility of eight inbred mouse strains to induction of SCC of the lung by skin painting with *N*-nitroso-tris-chloroethylurea and found that FVB/J mice were one of five strains in which squamous cell lung cancers occurred. In that study, FVB/J mice had an intermediate response. Similarly, Woodworth and colleagues (24) tested keratinocytes from newborn mice in vitro and found that FVB/N keratinocytes were 10 times more sensitive to chemically induced malignant conversion than were keratinocytes from other strains, a finding that was consistent with the known sensitivity of this strain to premalignant progression in vivo. In the Woodworth study (24), FVB/N keratinocytes formed tumors more frequently (64%) than SENCARA/Pt (31%), BALB/c (1.9%), or C57BL/6 (2.5%) keratinocytes, and, further, 78% of the tumors formed by FVB/N keratinocytes progressed to SCCs as compared with 46% for SENCARA/Pt-derived cells and < 3% for the cells from other strains. However, this predisposition to premalignant progression in FVB/N mice was not a dominant characteristic, because in F1 offspring of crosses from SENCARA/Pt and FVB/N mice, the frequency of malignant conversion reflected that in the SENCARA/Pt parent.

In conclusion, we report the development of SCCs of the skin at the sites of metallic ear tags composed of a nickel–copper alloy in almost 9% of our mouse population older than 300 days. The development of these cancers seems to be related directly to the presence of the ear tag, because they did not develop in the opposite ear, and they showed no association with the injection of a carcinogen (ENU) that is known to induce DNA mutations, or, as might be anticipated, with the presence of a lymphoma-associated human transgene expressed specifically in lymphocytes. All of the tumors that developed at the ear tag sites were diagnosed readily by morphologic criteria as being of squamous cell origin, and we detected no other kinds of cancers at ear tag sites in these mice. Most of these cancers were high-grade and led to premature removal of the animal. As discussed earlier, the FVB/N mouse strain is known to be particularly susceptible to the development of squamous cancers (11, 22, 24). We believe that the potentially carcinogenic chemical exposure from the ear tag over a prolonged period (especially from nickel, possibly combined with the additive effects of copper) in our incipient congenic FVB/N mouse strain provides a plausible explanation for the high incidence of development of SCCs in this mouse population.

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References

1. **al-Hiyasat, A. S., O. M. Bashabsheh, and H. Darmani.** 2002. Elements released from dental casting alloys and their cytotoxic effects. Int. J. Prosthodont. **15:**473-478.
2. **Baron, B. W., J. Anastasi, A. Montag, D. Huo, R. M. Baron, T. Karrison, M. J. Thirman, S. K. Subudhi, R. K. Chin, D. W. Felsher, Y.-X. Fu, T. W. McKeithan, and J. M. Baron.** 2004. The human *BCL6* transgene promotes the development of lymphomas in the mouse. Proc. Natl. Acad. Sci. USA **101:**14198-14203.
3. **Campo, M. S.** 1997. Bovine papillomavirus and cancer. Vet. J. **154:**175-188.
4. **Cangul, H., L. Broday, K. Salnikow, J. Sutherland, W. Peng, Q. Zhang, V. Poltaratsky, H. Yee, M. A. Zoroddu, and M. Costa.** 2002. Molecular mechanisms of nickel carcinogenesis. Toxicol. Lett. **127:**69-75.
5. **Chiu, A., A. J. Katz, J. Beaubier, N. Chiu, and X. Shi.** 2004. Genetic and cellular mechanisms in chromium and nickel carcinogenesis considering epidemiologic findings. Mol. Cell. Biochem. **255:**181-194.
6. **Faccioni, F., P. Franceschetti, M. Cerpelloni, and M. E. Fracasso.** 2003. In vivo study on metal release from fixed orthodontic appliances and DNA damage in oral mucosa cells. Am. J. Orthod. Dentofacial Orthop. **124:**687-693.
7. **Felsher, D. W. and J. M. Bishop.** 1999. Reversible tumorigenesis by *MYC* in hematopoietic lineages. Mol. Cell **4:**199-207.
8. **Forslund O., B. Lindelof, E. Hradil, P. Nordin, B. Stenquist, R. Kirnbauer, K. Slupetzky, and J. Dillner.** 2004. High prevalence of cutaneous human papillomavirus DNA on the top of skin tumors but not in "stripped" biopsies from the same tumors. J. Invest. Dermatol. **123:**388-394.
9. **Friedman, K. E. and S. E. Vernon.** 1983. Squamous cell carcinoma developing in conjunction with a manibular staple bone plate. J. Oral Maxillofac. Surg. **41:**265-266.
10. **Helfrich, I., M. Chen, R. Schmidt, G. Fürstenberger, A. Kopp-Schneider, D. Trick, H.-J. Gröne, H. zur Hausen, and F. Rösl.** 2004. Increased incidence of squamous cell carcinomas in *Mastomys natalensis* papillomavirus E6 transgenic mice during two-stage skin carcinogenesis. J. Virol. **78:**4797-4805.
11. **Hennings, H., A. B. Glick, D. T. Lowry, L. S. Krsmanovic, L. M. Sly, and S. H. Yuspa.** 1993. FVB/N mice: an inbred strain sensitive to the chemical induction of squamous cell carcinomas in the skin. Carcinogenesis **14:**2353-2358.
12. **Karras, P. J., M. Barawi, B. Webb, and A. Michalos.** 1999. Squamous cell papillomatosis of esophagus following placement of a self-expanding metal stent. Dig. Dis. Sci. **44:**457-461.
13. **Kasprzak, K. S., F. W. Sunderman, Jr., and K. Salnikow.** 2003. Nickel carcinogenesis. Mutat. Res. **533:**67-97.
14. **Kawanishi, S., Y. Hiraku, M. Murata, and S. Oikawa.** 2002. The role of metals in site-specific DNA damage with reference to carcinogenesis. Free Radic. Biol. Med. **32:**822-832.
15. **Kim, S.-H., K.-S. Kim, E.-J. Lee, M.-O. Kim, J.-H. Park , K-I. Cho, K. Imakawa, B.-H., Hyun, K.-T. Chang, H. T. Lee, and Z.-Y. Ryoo.** 2004. Human keratin 14 driven HPV 16 E6/E 7 transgenic mice exhibit hyperkeratosis. Life Sci. **75:**3035-3042.
16. **Nieminen, T. M.** 2004. Effects of soil copper and nickel on survival and growth of Scots pine. J. Environ. Monit. **6:**888-896.
17. **Quereux, G., J. M. N'Guyen, and B. Dreno.** 2004. Human papillomavirus and extragenital in situ carcinoma. Dermatology **209:**40-45.
18. **Roscoe, D. E., L. R. Veikley, M. Mills, Jr., and L. Hinds 3rd.** 1975. Debilitating ossifying fibromas of a white-tailed deer associated with ear tagging. J. Wildl. Dis. **11:**62-65.
19. **Sundberg, J. P., R. J. Chiodini, and S. W. Nielsen.** 1985. Transmission of the white-tailed deer cutaneous fibroma. Am. J. Vet. Res. **46:**1150-1154.
20. **Sundberg, J. P., E. S. Williams, D. Hill, W. D. Lancaster, and S. W. Nielsen.** 1985. Detection of papillomaviruses in cutaneous fibromas of white-tailed and mule deer. Am. J. Vet. Res. **46:**1145-1149.
21. **Waalkes, M. P., S. Rehm, K. S. Kasprzak, and H. J. Issaq.** 1987. Inflammatory, proliferative, and neoplastic lesions at the site of metallic identification ear tags in Wistar [(Crl:WI)BR] rats. Cancer Res. **47:**2445-2450.
22. **Wang, Y., Z. Zhang, Y. Yan, W. J. Lemon, M. LaRegina, C. Morrison, R. Lubet, and M. You.** 2004. A chemically induced model for squamous cell carcinoma of the lung in mice: histopathology and strain susceptibility. Cancer Res. **64:**1647-1654.
23. **Wong, C. K. and A. P. Pak.** 2004. Acute and subchronic toxicity of the heavy metals copper, chromium, nickel, and zinc, individually and in mixture, to the freshwater copepod *Mesocyclops pehpeiensis*. Bull. Environ. Contam. Toxicol. **73:**190-196.
24. **Woodworth, C. D., E. Michael, L. Smith, K. Vijayachandra, A. Glick, H. Hennings, and S. H. Yuspa.** 2004. Strain-dependent differences in malignant conversion of mouse skin tumors is an inherent property of the epidermal keratinocyte. Carcinogenesis **25:**1771-1778.
25. **Wozniak, K. and J. Blasiak.** 2004. Nickel impairs the repair of UV- and MNNG-damaged DNA. Cell Mol. Biol. Lett. **9:**83-94.