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Subcutaneous microchip-associated tumours in B6C3F1 mice: A retrospective study to attempt to determine their histogenesis

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Abstract

Fifty-two subcutaneous tumours associated with microchip were collected from three carcinogenicity B6C3F1 mice studies. Two of these 52 tumours were adenocarcinoma of the mammary gland located on the dorsal region forming around the chip. All the other 50 were mesenchymal in origin and were difficult to classify on morphological grounds with haematoxylin–eosin. These sarcomas were investigated using a panel of immunohistochemistry and special stains consisting of desmin, smooth muscle actin (SMA), myogenin, S100, mouse macrophages, phosphotungstic acid haematoxylin (PTAH) and Masson's trichrome (MT). All the sarcomas displayed the same histochemical characteristics and a close immunophenotype, characterized by desmin±, SMA+, myogenin−, S100−, mouse macrophages+ and PTAH−. These tumours thus appear to have similar histologic-type lineage and designation as sarcomas not otherwise specified (NOS) with a large myofibroblastic component appears today to be more appropriate and it is likely to clarify them in the future with the emergence of new markers.

Keywords: Microchip implant; Transponder; Foreign-body tumour; Induced-sarcoma; Histogenesis; Immunohistochemistry; Malignant fibrous histiocytoma; Sarcoma NOS

Introduction

Implantable microchips were introduced in the late 1980s and have been used routinely for more than 10 years in long-term rodent toxicity and carcinogenicity studies to identify animals. The system is based on radiofrequency identification (RFID) technology (Weyand, 1998). The materials consist of a microchip transponder which is implanted in the subcutaneous tissue of the animal, a scanner which reads the unique preprogrammed microchip 10–15 digit code number and an optional digital display which acts as a computer interface for the data downloading. The microchip transponder is hermetically sealed in a cylindrical inert glass capsule measuring 12 mm in length and 2 mm in diameter and partially covered on a length of 5 mm by a porous polypropylene polymer sheath as an antimigration measure. These are easily implanted subcutaneously in the mid-dorsal region using the individual sterile-needle assembly in which the microchip is packaged. It is then interrogated by a very low power radio-frequency signal emitted by the scanner and transmits the encoded identifying number to a "reader" that interprets the

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number and transfers it to a computer (Ball et al., 1991; Elcock et al., 2001; Rao and Edmondson, 1990; Sacco, 1992).

Compared with the other conventional means such as ear notches or tags, toe-clips or tattoos which can be lost, painful or become hard to see (Sacco, 1992; Tillmann et al., 1997; Weyand, 1998) the implantable microidentification (IMI) is recommended as a fast, secure and durable means of animal identification. Two studies were performed in rodents using 40 Sprague–Dawley rats (Ball et al., 1991) and a 2-year study performed on 140 B6C3F1 mice (Rao and Edmondson, 1990). Both studies reported no adverse clinical or histopathological effects associated with the microchips and only a few cases of loss of identification at the end of the study. This lack of side effects has been confirmed in other articles in several species (Mrozek et al., 1995; Murasugi et al., 2003). In addition to an easy animal identification, the microchip can be retained with the fixed tissues giving a permanent identification. The computer read out of the number reduces the human errors in the data registration and therefore IMI are considered one of the most effective methods and have become a widespread system of animal identification.

One of the most potentially serious disadvantages of the microchip implantation is the possibility that foreign-body-induced tumours may develop in long-term rodent studies. Such tumours have been described in one recent article in F344 rats (Elcock et al., 2001) and in publications in various laboratory mouse strains (Blanchard et al., 1999; Johnson, 1996; Palmer et al., 1998; Tillmann et al., 1997). The tumours were all diagnosed as malignant and mesenchymal in origin. In F344 rats, they were noted in order of frequency as malignant schwannoma, fibrosarcoma, anaplastic sarcoma and histiocytic sarcoma confirmed by immunochemistry (Elcock et al., 2001). In mice (strain not identified), these sarcomas were described as typical foreign-body-induced sarcomas, generally fibrosarcomas (Johnson, 1996), or in CBA/J mice (Tillmann et al., 1997) as fibrosarcoma and malignant fibrous histiocytoma and in B6C3F1 mice (Palmer et al., 1998) as fibrosarcoma, and in heterozygous $p53^{+/-}$ transgenic mice (Blanchard et al., 1999) as undifferentiated sarcomas. In none of these studies in mice was there any confirmation of this morphological diagnosis by other methods (e.g. electron microscopy or immunohistochemistry).

At MDS Pharma Services, microchip-associated tumours have been observed for several years in 2-year carcinogenicity mice studies. It is recognized that it is difficult to categorize these sarcomas with routine evaluation and that their histological diagnosis based on morphology alone remains subjective even according to the well-established diagnostic criteria for soft tissue tumours in rodents (Greaves, 1997; Heider et al., 1994).

This review of 52 subcutaneous tumours associated with microchip was conducted in order to more fully define these tumours by use of immunohistochemistry and special stains in B6C3F1 mice which had been used in 2-year carcinogenicity studies.

Material and methods

Study population and sample collection

Formalin-fixed tissue from subcutaneous microchip-associated tumours from three 2-year oral carcinogenicity mice studies performed at MDS Pharma Services (Les Oncins, 69210 Saint Germain s/ L'Arbesle, France) were used.

The three carcinogenicity studies were conducted in B6C3F1 mice from Charles River Laboratories (9801 Shavor Road, Portage, Michigan, USA). Two studies consisted of five groups in each sex; two control groups and three treatment groups (low, mid and high doses). In one study, the group size was 55 and 60 in the other. The third carcinogenicity study was an internal study to provide background data for B6C3F1 mice in these laboratories and had two control groups of 55 mice/sex. These studies were conducted to meet international regulatory and ethical norms. Animals were acclimatised for 2 weeks prior to the start of the study. All the 1260 mice were identified by the subcutaneous implantation of microchips (BioMedic Data System Inc., European distributor PLEXX BV, Elst, The Netherlands) a few days prior to initiation of studies. The microchip measured $2 \times 12\,mm^2$, was prepackaged in the lumen of a sterile 12-gauge needle and related to an implantation device supplied by the manufacturer. The subcutaneous implantations were performed dorsolaterally in the back. At the start of the studies, mice were 6–8 weeks old. During the studies, they were kept under the same standard laboratory conditions (air-conditioned building with a barrier protected unit; room temperature $22 \pm 2\,^{\circ}C$; relative humidity $55 \pm 15\%$; minimum 15 air changes/h, light–dark cycle 12–12 h). All were housed singly in stainless-steel mesh cages ($225 \times 95 \times 125\,mm^3$). Mice were fed with pelleted complete diet ad libitum (A04-C10, Usine D'alimentation Rationnelle, Villemoisson, 91 360 Epinay s/Orge, France), sterilised by irradiation and analysed for the absence of chemical and bacteriological contaminants. Tap water was filtered (0.2 μm) and distributed ad libitum and was analysed once a year for chemical and at least twice a year for bacterial contaminants (Laboratoire Santé Environnement Hygiène de Lyon). A full clinical examination was performed once every 4

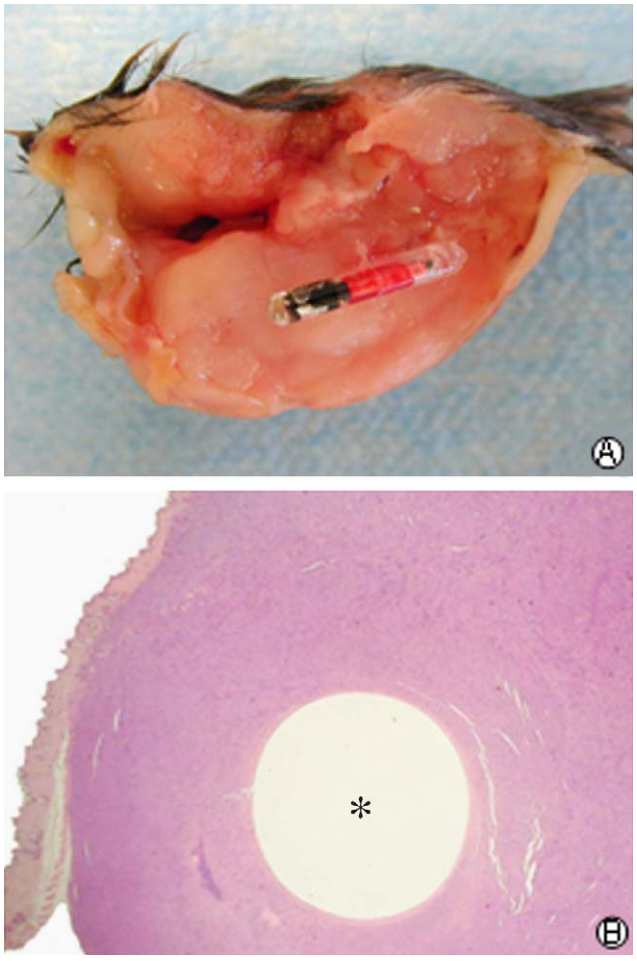


Fig. 1. Gross and microscopic appearance of a microchip-associated tumour. (A) The microchip has been removed from the cavity where it resided in situ (size of microchip 2×12 mm). (B) Cavity related to the removed microchip (asterisk) (H&E, $\times 25$).

weeks up to week 26, once weekly thereafter, and mortality checks were performed daily throughout the study. Animals were examined for grossly visible or palpable masses at the time of the clinical examination, recording the time of onset, location, size and appearance of masses. Individual body weight and food consumption were recorded weekly for the first 16 weeks, and once a month thereafter. All animals including any found dead or moribund were submitted to full necropsy procedures. Mice found moribund during the studies and those that survived to the end of the study were euthanized by CO_2 asphyxiation followed by exsanguination. Necropsies were performed and included a complete external and internal examination. Major organs were weighed and a full list of tissues including gross abnormalities were collected and fixed in 10% buffered formalin. After fixation, tissues were trimmed and processed, embedded in paraffin wax, sectioned at 4–6 μm and stained with haematoxylin and eosin (H&E). Slides were then evaluated by a pathologist and peer-reviewed by another pathologist. Tumours were diagnosed according to standardized nomenclature [10,13]. Both necropsy and histopathological data were collected using a computerized data capture system.

To select the tissues for this review the data from the original studies were analyzed to identify masses either obviously or possibly related to the microchip. After evaluation of the data and the original sections (Fig. 1B), a selection of tumours was defined (Table 1). The residual fixed tissues from the studies were resampled and new paraffin blocks were prepared. The fixation time of the tumours differed according to which study they had come from; for studies 1 and 2 this was some 8 months, and for study 3 this was over 4 years.

Table 1. Distribution of the microchip-associated subcutaneous tumours in the three oral carcinogenicity B6C3F1 mice studies according to sex, dose-level and diagnosis on morphological grounds

H&E diagnosis	Study 1 ($n = 55$ mice/sex/group)										Study 2 ($n = 55$ mice/sex/group)				Study 3 ($n = 60$ mice/sex/group)									
	Males					Females					Males		Females		Males					Females				
	1	2	3	4	5	1	2	3	4	5	1		1		1	2	3	4	5	1	2	3	4	5
Fibrosarcoma	1	1				1	1	1	1	1	1				1		1	1	1	2		1	1	1
Rhabdomyosarcoma	1	3	1			1	1	1					3					1						
Leiomyosarcoma							1										1							
MFH	1							1																1
NOS Sarcoma	1	1	3	2	3	2		1	1							1		1						
Mammary gland adenocarcinoma								1	1															
Total/sex			18					16			1		3				8					6		
Total													52											

Groups 1 and 5 were vehicle controls. The others were ascending dosages.
MFH = malignant fibrous histiocytoma; NOS = not otherwise specified.

Immunohistochemistry

The selected tumours were reacted for five different immunomarkers using the labelled avidin–biotin technique. After section at 4–6 μm, the slides were dried at 37° for 30 min, deparaffinized and rehydrated. Heat-Induced Epitope Retrieval (HIER) was performed manually in 0.01 mol/L of citrate acid buffer twice for 5 min in the microwave at 750 W. Slides were allowed to return to room temperature, then placed in an automate stainer (Lab Vision autostainer #480-2D). The machine was programmed to perform pretreatment of the slides firstly an endogenous peroxide block with 3% hydrogen peroxide for 30 min (Sigma #6520) then a 10 min blocking of endogenous avidin followed by a similar blocking of biotin (Dako #X0590), finally a 30-min treatment with normal goat serum diluted at 5% (Dako #X0907). The slides were then incubated for 1 h with the primary antibody at room temperature (Table 2), followed by incubation for 30 min with goat anti-rabbit/mouse biotinylated secondary antibodies followed by Strept AB Complex/HRP for 30 min (Dako #K0492). Finally, the slides were treated with the chromogen DAB (3,3′-Diaminobenzidine Tetrahydorchloride benzidine-Dako #PA-125-HD) during 2–5 min. The slides were then counterstained with haematoxylin and mounted.

Polyclonal primary antibodies were used as there was less likelihood of background since most of the available monoclonals were mouse derived. In addition, the use of the same polyclonal antibody type produced in rabbits gave a constant program for the automated staining.

The cross-reactivity for each primary antibody used was confirmed by staining control mouse tissues fixed during 24 h. The stability of the antigens was verified by staining control tissue fixed for an equal time as the tumours of the studies were.

Special stains

In addition to immunohistochemical investigations, two special stains were performed on the 50 sarcomas. Phosphotungstic acid-haematoxylin (PTAH) stain was used to demonstrate any cytoplasmic myofibrils of myogenic tumour cells (cross-striations in rhabdomyosarcomas and longitudinal myofibrils in leiomyosarcomas) and Masson's trichrome (MT) stain to evaluate the amount and the distribution of mature collagen.

Results

Data evaluation

In all three carcinogenesis studies, the overall occurrence of the microchip-associated tumours was 4.1% with 52 animals bearing a microchip-associated tumour out of 1260. Some differences were noted from one study to another; 34/550 (6.2%), 4/110 (3.6%) and 14/600 (2.3%) (Table 1). As these were only sampled and examined histologically when gross abnormalites were noted, it is possible that early reaction could have been missed. These incidences may therefore slightly underestimate the true occurrence.

Within the three studies, there were 27 males and 25 females bearing a microchip-associated tumour out of the 630 mice of each sex (Table 1); therefore, there was no sex-related trend in these tumours. In addition, they were distributed randomly across the control and treated groups without a test item-related trend (Table 1).

Clinically, all microchip-associated tumours appeared during the second year of the studies, except for one tumour detected on week 51 in one female. The mean in-life onset was 90 weeks. Most of the animals (33/52 = 65.4%) with microchip-associated tumours died prematurely; 28/33 mice were sacrificed for ethical reasons due to the size of the masses, and in 5/33 cases the deaths were spontaneous and attributed to the masses. The death of the mice or the sacrifices occurred from 1 to 7 weeks after the first in-life observation, with a mean of 4

Table 2. List of the five primary antibodies

Primary antibodies	Main reactivity	Antibody type	Providers and references	Dilutions
Anti-desmin	Myogenic tumors; RMS and LMS	Rabbit polyclonal	Abcam ab8592	1:50
Anti-alpha smooth muscle actin	LMS; Myofibroblastic lesions	Rabbit polyclonal	Abcam ab5694	1:200
Anti-myogenin	RMS	Rabbit polyclonal	Santa Cruz biotechnology sc-576	1:200
Anti-mouse macrophages	Macrophages; MFH	Rabbit Polyclonal	Acris Antibodies CL194P	1:50
Anti-S100	Broad; schanommas	Rabbit Polyclonal	Dako Z0311	1:200

RMS = rhabdomyosarcoma; LMS = leiomyosarcoma; MFH = malignant fibrous histiocytoma.

weeks. These masses were late developing but showed a rapid increase in size.

In all three studies, these microchip-associated tumours only contributed 2.6% (33 of the 1260 mice) to the loss of animals during the studies.

Grossly, all the masses contained the microchip (Fig. 1A) or were adjacent to it. 80.7% masses were located at the implantation site (dorsal, thoraco-dorsal, lumbo-dorsal and thoracic regions). The other 19.3% tumours were located in the region of the limbs (4/52), the abdominal region (4/52) or dorsal head (1/52) which confirm the potential for the microchip to migrate from the implantation site. Grossly, all the microchip-associated tumours had a similar appearance consisting

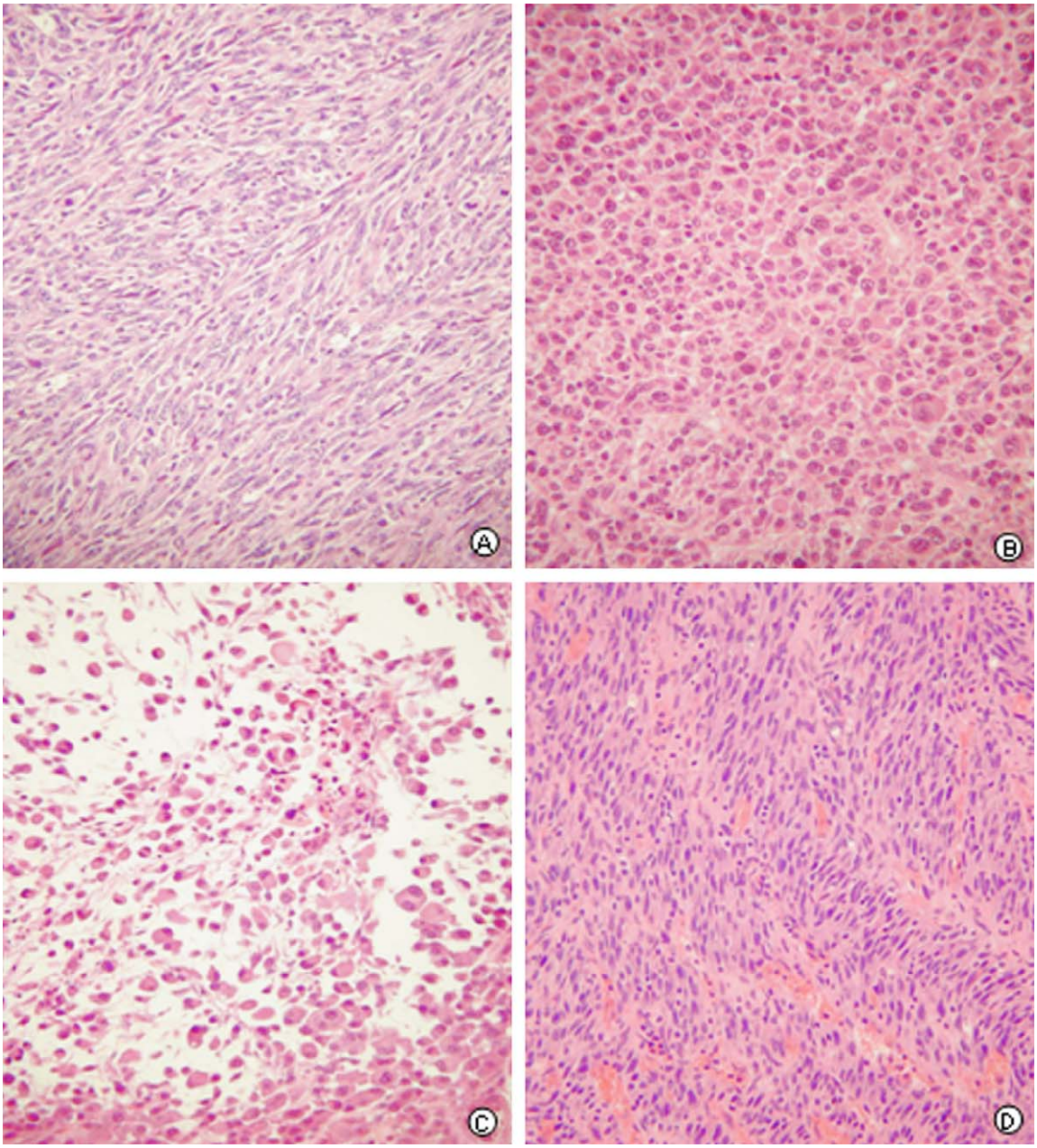
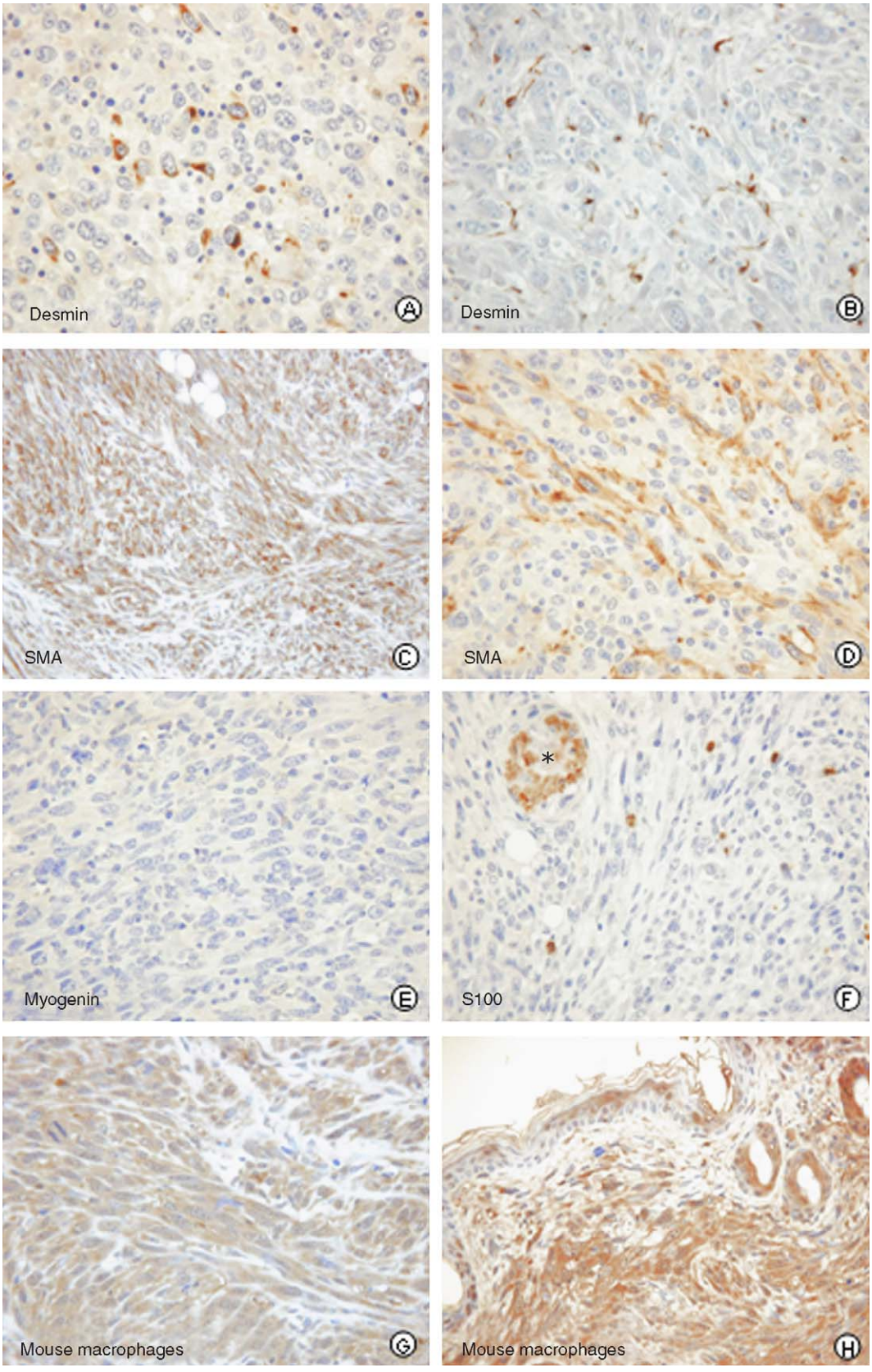


Fig. 2. Histological appearance of microchip-associated tumours (H&E, × 250). (A) Sarcoma characterized by fascicular pattern of relatively monomorphic fusiform spindle cells with oval nuclei often separated by scant collagen fibers; firstly diagnosed as fibrosarcoma on morphological grounds. (B) Sarcoma characterized by sheets of round to polygonal cells associated to binucleated cells with excentric nuclei and/or multinucleated giant cells; morphological features suggesting a rhabdomyosarcoma. (C) Sarcoma characterized by two distinct cellular populations; fibroblastic and histiocyte-like cells, suggesting a malignant fibrous hitiocytoma. (D) Sarcoma characterized by interwoven fascicles of long spindle-shaped eosinophilic cells with blunt-ended oval hyperchromatic nuclei not separated by collagenous fibers; firstly diagnosed as leiomyosarcoma on morphological grounds.


Desmin
A
Desmin
B
SMA
C
SMA
D
Myogenin
E
*
S100
F
Mouse macrophages
G
Mouse macrophages
H

of a solid beige mass variable in size with a mean of $3 \times 2.5 \times 1.7\,cm^3$ in diameter, frequently immovable, occasionally multinodular and sometimes ulcerated.

The routine microscopical examination showed 50 of the 52 tumours to be mesenchymal in origin and two to be adenocarcinomas of mammary gland located at the dorsal posterior region. All sarcomas were characterized by a poorly delineated, non-encapsulated, densely cellular mass, located in the subcutis but frequently infiltrating the panniculus muscle and various layers of the skin with occasional ulcerations. A round-to-oval empty space of 2 mm diameter corresponding to the cast of the microchip was frequently seen and associated with a vestigial fibrous capsule and/or a focus of necrosis (Fig. 1B). Neoplastic cells were arranged in a variety of patterns, including fascicular, storiform, myxoid or solid sheets. In most instances, several mixed patterns were present in the same tumour. Neoplastic cells consisted of pleomorphic, polygonal, round or spindle-shaped cells having pleomorphic, cigar-shaped to round sometimes bizarre large and/or multilobulated, hyper- or hypochromatic vesiculated nuclei often with one or more prominent nucleoli. In some tumours, there were multinucleated giant cells with slight-to-large amount of amphophilic cytoplasm with some marked nuclear atypia. The mitotic index for the tumor cells was moderate (1–2 mitosis/high-power field) to high (5–9 mitosis/high power-field). The fibrovascular stroma was discrete in the small tumours and more prominent in bigger ones, especially close to the inner capsule where it was often seen as a cavernous network. Small thin capillaries were visible diffusely in all tumours. Areas of haemorrhage and necrosis with variable amounts of inflammation were common in the larger tumours. Although these tumours shared many histological features, they were diagnosed following the H&E examination according to well-established diagnostic criteria for soft tissue tumours in rodents (Greaves, 1997; Heider and Eustis, 1994). Some were diagnosed as fibrosarcoma when tumor cells were predominantly spindle-shaped, relatively monomorphic often separated by thin collagenous fibers and arranged in interlacing bundles with occasional "herring-bone" disposition (Fig. 2A). Others were diagnosed as rhabdomyosarcoma when the predominant pattern was unorganised solid sheets of various proportions of large pleomorphic strap-like cells with modest to abundant eosinophilic to basophilic cytoplasm, multinucleated giant cells and/or round cells with one or two eccentrically located nuclei, and sometimes separated by a few interlacing bundles of spindle-shaped cells occasionally containing central rows of nuclei (Fig. 2B). Others were diagnosed as malignant fibrous histiocytoma with two different cell populations, one fibroblastic and one histiocyte-like (Fig. 2C). Only two of this group of tumours were diagnosed as leiomyosarcoma because of the predominant long spindle-shaped eosinophilic cells with blunt-ended oval and often hyperchromatic nuclei (Fig. 2D). Sarcomas judged not sufficiently differentiated to be classified at the H&E evaluation, were identified as sarcomas not otherwise specified (NOS).

Microscopic metastases were detected in four mice; two had metastases in the lungs only; one in the lungs and liver and another in the wall of the stomach and in the pancreas.

Immunohistochemistry

Preliminary immunohistochemistry investigation indicated that there was no influence of the fixation time in neutral buffered formalin on the different antigens. In a few cases, the background staining prevented interpretation and these cases were excluded from the results. For each antibody tested, positive tumours were reported in four categories according to the proportion of positive tumour cells — less than 10% (+), 10–30% (++), 30–50% (+++) and more than 50% (++++).

Desmin

Positive immunostaining of tumour cells was seen in 22/47 (46.8%) tumours examined. Positive reaction was seen in either diffusely distributed individual cells (Fig. 3B) or multifocally (Fig. 3A). In 11 of these 22 tumours (50%), positive desmin expression was seen in less than 10% of the tumour cells. In 8/22 tumours (36.3%), positive desmin expression was mostly multifocal and present in 10–30% of the tumour cells, while in 3/22 tumours (13.6%) the staining was multifocal or diffuse and present in 30–50% of the tumour cells.

Desmin staining also confirmed the infiltration of the panniculus muscle in many tumours and the extensive cavernous network of capillaries within the tumour, especially around the hole left by the microchip.

Fig. 3. Immunohistochemical stainings (A, B, D, E, F and G: ×400; C and H: ×250). (A) Diffuse desmin expressed by less than 10% of the tumour cells. (B) Focal desmin expression by a cluster of tumour cells. (C) Illustration of SMA expression by more than 50% of the tumour cells. (D) SMA was expressed predominantly in the cytoplasm of the spindle-shaped cells. (E) All tumours were negative for myogenin. (F) All tumors were negative for S100 (asterisk: positive nerve); a positive immunoreactivity was however observed in small round non-neoplastic cells diffusely in the tumour and were identified as dentritic cells and/or macrophages. (G) Cytoplasm staining for mouse macrophages is present in more than 80% of the tumour cells. (H) Illustration of the non-specificity of the anti-mouse macrophages primary antibody. A staining of the epidermal and follicular cells can easily be seen.

Alpha smooth muscle actin (SMA)

Positive immunostaining was seen in tumour cells in 41/47 (87.2%) of the tumours examined. In 4/41 (9.7%) SMA expression was present in less than 10% of tumour cells, in 11/41 (26.8%) it was 10–30%, in 12/41 (29.2%) this was 30–50% and in 14/41 (34.1%) this was seen in more than 50%. SMA activity was principally observed in fusiform spindle cells (Fig. 3C and D), and the percentage of positive tumour cells correlated with the number of spindle-shaped cells in the different tumours.

Myogenin

All the sarcomas examined including the tumours initially diagnosed as rhabdomyosarcomas, were negative (Fig. 3E).

S-100

Tumour cells of all sarcomas were negative. It was however noted that non-neoplastic individual small round cells diffusely distributed among the neoplastic cells or occasionally localized in the blood vessels were positive (Fig. 3F).

Mouse macrophages

All the sarcomas examined exhibited positive immunostaining. In 43/45 (95.5%) of these tumours over 80% of the tumour cells stained positive. These included spindle, round and pleomorphic cells (Fig. 3G). Two of the tumours only showed a positive reaction in 30–50% of the tumour cells. This antibody showed some lack of specificity, as positive reactions were observed in epidermal and follicular epithelium, in epithelial cells of the sebaceous glands and occasionally in skeletal muscle fibers (Fig. 3H).

Diagnoses recorded after the first H&E evaluation are presented in Table 3, along with their similar immunophenotype.

Special stains

There were no cytoplasm cross-striations or longitudinal myofibrils detected either with PTAH or MT stains in any tumour cells of the 50 sarcomas examined.

MT stain confirmed that the capsule which was often formed immediately adjacent to the microchip was

Table 3. Initial H&E tumour diagnosis and its corresponding immunohistochemistry

		Desmin	SMA	Myogenin	S100	Mouse macrophage
Fibrosarcoma	−	10/15 (66.6%)	3/16 (18.7%)	17/17 (100%)	17/17 (100%)	
($n = 17$)	+	4/15 (26.6%)				
	++	1/15 (6%)	3/16 (18.7%)			
	+++		3/16 (18.7%)			
	++++		7/16 (46.6%)			16/16 (100%)
Rhabdomyosarcoma	−	6/12 (50%)	1/12 (8.3%)	12/12 (100%)	12/12 (100%)	
($n = 12$)	+	2/12 (16.6%)				
	++	3/12 (25.1%)	4/12 (33.3%)			
	+++	1/12 (8.3%)	5/12 (41.6%)			1/10 (10%)
	++++		2/12 (16.6%)			9/10 (90%)
Leiomyosarcoma	−	2/2 (100%)		2/2 (100%)	2/2 (100%)	
($n = 2$)	+					
	++					
	+++		1/2 (50%)			
	++++		1/2 (50%)			2/2 (100%)
MFH	−			3/3 (100%)	3/3 (100%)	
($n = 3$)	+	1/3 (33.3%)	1/3 (33.3%)			
	++	1/3 (33.3%)				
	+++	1/3 (33.3%)	1/3 (33.3%)			1/3 (33.3%)
	++++		1/3 (33.3%)			2/3 (66.6%)
NOS Sarcoma	−	7/15 (46.6%)	2/14 (14.3%)	16/16 (100%)	15/15 (100%)	
($n = 16$)	+	4/15 (26.6%)	3/14 (21.4%)			
	++	3/15 (20%)	4/14 (28.6%)			
	+++	1/15 (6.6%)	2/14 (14.3%)			
	++++		3/14 (21.4%)			14/14 (100%)

−: no positive tumour cells; +: 0–10% positive tumour cells; ++: 10–30% positive tumour cells; +++: 30–50% positive tumour cells; ++++: more than 50% positive tumour cells.

MFH = malignant fibrous histiocytoma; NOS = not otherwise specified.

Immunostaining considered to be doubtful or non-interpretable were excluded from the results.

composed of mature collagen. This stain also revealed that fibers of mature collagen were especially numerous in the areas of spindle-shaped cells, but did not show any significant difference in the amount and distribution of the mature collagen fibers between the different types of sarcomas.

Discussion

Tumours associated with the identification microchips have been reported in long-term chronic toxicity/carcinogenicity rodents studies; once in rats (Elcock et al., 2001) and in some reports in various laboratory mouse strains, including an indefinite strain (Johnson, 1996), the CBA/J (Tillmann et al., 1997), B6C3F1 (Palmer et al., 1998) and in heterozygous $p53^{+/-}$ transgenic mice (Blanchard et al., 1999). Recently, a liposarcoma associated with microchip has been described in one domestic dog (Vascellari et al., 2004). To our knowledge, microchip-associated tumours in other species have not yet been reported.

In this review, the mean occurrence of these tumours (4.1%) was slightly higher than the reported data for the non-genetically modified mice; 2% for B6C3F1 mice (Palmer et al., 1998), less than 1% for an unidentified strain (Johnson, 1996) and 1.2% for the CBA/J females and 0.5% for the CBA/J males (Tillmann et al., 1997). Our results taken with this published data supports the hypothesis that there is a difference in susceptibility between strains. This was previously suggested because of the absence of microchip-associated tumours in the CD-1 strain (Elcock et al., 2001; Palmer et al., 1998).

In this retrospective evaluation, two adenocarcinomas of the mammary gland localised on the dorsal region which were seen to include the microchip have been reported. As the extent of the mouse mammary tissue shows an individual variability, the fact that these two tumours contained the microchip is probably incidental. The extensive (50 sarcomas) and varied application of immunohistochemical and special stains to aid the determination of the histogenesis has not been previously performed in mice. The only previous report including immunohistochemical investigation was on eight sarcomas from F344 rats (Elcock et al., 2001).

Immunohistochemical markers and special stains were chosen to attempt to identify the histogenesis of the sarcomas found. Desmin and alpha smooth muscle actin (SMA), both muscular markers, were performed to differentiate rhabdomyosarcomas (desmin+ and SMA−) from leiomyosarcomas (desmin± and SMA+). As neither of these markers is sufficiently specific (Cerilli and Wick, 2002; Coindre, 2003; Hasegawa et al., 2003; Kodet, 1989; Ordonez, 1998; Truong et al., 1990), they were complemented by myogenin, a muscular marker considered as extremely sensitive and reported as specific for identifying human rhabdomyosarcomas (Cessna et al., 2001; Coindre, 2003; Cui et al., 1999; Kumar et al., 2000). It was shown (Coindre, 2003) that more than 90% of human rhabdomyosarcomas are positive for myogenin and that the non-rhabdomyosarcomas are all clearly negative (Kumar et al., 2000) or only show rare focal reactivity (Cessna et al., 2001). Myogenin belongs to a group of myogenic regulatory proteins whose expression determines commitment and differentiation of primitive mesenchymal cells into skeletal muscle and is also expressed in regenerative skeletal muscle cells. As no confirmed rhabdomyosarcoma in mice were available to use as positive control, regenerative skeletal muscle from an independent study was used. In addition to muscular immunomarkers, PTAH stain was performed to enhance eventual myofibrils in the cytoplasm of myogenic tumour cells (cross-striated in rhabdomyosarcoma and longitudinal in leimyosarcoma). The clearly negative activity of the myocyte markers, in particular, myogenin showed that none of the microchip-associated sarcomas was a rhabdomyosarcoma. This finding was confirmed by the negativity of PTAH stain for all tumours. As shown in Table 3, the tumours previously diagnosed (with H&E) as rhabdomyosarcomas did not show any greater activity of desmin than the other types of sarcomas confirming that these tumours were not rhabdomyosarcomas and that desmin is not a specific marker for this type of tumour. In humans, desmin can be expressed in tumour-associated desmoplasia, myofibroblasts of the granulation tissue and some reactive dentritic cells (Coindre, 2003; Truong et al., 1990). In our survey, 46.8% of tumours showed desmin-positive cells, however, less than 10% of the tumour cells were marked in the majority of the tumours. This could be explained by the lack of specificity but also by the difficulties in distinguishing positive cells of the neovascularisation from spindle tumour cells, thus overestimating the percentage of the tumour cells showing activity. Both tumours previously diagnosed on morphological grounds as leiomyosarcoma (using H&E) were highly positive for SMA but negative for PTAH and desmin suggesting that these and the other sarcomas were not of myogenic origin.

The SMA positive activity in 80% of the tumours examined indicated the lack of specificity of this marker for smooth muscle tumours. Coindre reported that SMA should be interpreted with caution because it could be positive in many non-muscular/myofibroblastic lesions (Coindre, 2003). As SMA activity was mainly observed in long fusiform spindle cells, this is possibly due to myofibroblastic differentiation of the tumour cells and/or the presence of reactive myofibroblasts. SMA alone was thus not considered useful in differentiating mesenchymal sarcomas.

Although none of these tumors was originally diagnosed as a malignant schwannoma, S100 was performed as malignant schwannomas were reported as the most common tumour associated with microchip in F344 rats (Elcock et al., 2001). Tumour cells of all the sarcomas in this present survey were clearly negative for S100, further indicating that they were not malignant schwannomas. The S100-positive non-neoplastic cells seen diffusely among the neoplastic cells are most probably intratumoral dendritic cells. Recently, it was however reported that S100 was not sufficient in itself to differentiate dentritic cells and macrophages in formalin-fixed tissue sections (Vakkila et al., 2005).

Malignant fibrous histiocytoma (MFH) is a histologically heterogeneous group of sarcomas whose histogenesis has been a matter of debate for many years because of its various cellular components. However, it is now well-recognized in man that the histiocyte-like components are reactive macrophages infiltrating the tumour and that the tumour cells themselves belong to a fibroblastic lineage differentiated from mesenchymal progenitor cells (Gazziola et al., 2003; Hatano et al., 1999; Takeya et al., 1995; Wood et al., 1986). As mice and human MFH are reported to have many similarities (Schneider et al., 1999), immunostaining for mouse macrophages was performed in this survey. Staining for mouse macrophages was positive for all microchip-associated sarcomas examined and involved over 80% of the tumour cells in the majority of tumours. Positive cells were either histiocyte-like (round/oval and/or pleomorphic cells) and spindle-shaped without any differences in the staining intensity between the different cellular morphologies. In addition, positive staining was seen in various non-tumour cells. This lack of specificity of the mouse macrophage marker does not allow its use in identifying the non-neoplastic histiocytes in MFH. The positivity of the majority of tumour cells indicates that a common antigen expression may be present but could also indicate a lack of specificity. A specific cell type designated as ''fibrohistiocytoid cells'' or metamorphosed fibroblasts was described in various chronic inflammatory processes and in MFH in man as a cell with morphological, enzyme histochemical and immunohistochemical characteristics intermediate between fibroblasts and histiocytes (Imai et al., 1989). In a recent experimental murine model, it was concluded that the ''fibrohistiocytoid cells'' were present in MFH not only as merely morphologically changed fibroblasts but actually contributed to the development of MFH in mice (Osanai et al., 2000). However, the latest World Health Organization (WHO) classification of soft tissue tumours in man (Fletcher et al., 2002) by the incorporation of the immunohistochemical results into the diagnostic criteria no longer includes MFH as a distinct diagnostic category but rather as subtypes of undifferentiated pleomorphic sarcomas (sarcomas NOS), which leads to the morphologic diagnosis of MFH in man being abandoned (Randall et al., 2004).

The differentiation of the tumours of this survey on purely morphological grounds is difficult as they appear to have similar histologic-type lineage, as illustrated by their immunohistochemical profile. Designation as NOS sarcoma with a large myofibroblastic component appears to be more appropriate. These sarcomas are likely to be better specified in the future with the emergence of new specific markers.

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