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Transponder-Induced Sarcoma in the Heterozygous $p53^{+/-}$ Mouse

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ABSTRACT

Heterozygous $p53^{+/-}$ transgenic mice are being studied for utility as a short-term alternative model to the 2-yr rodent carcinogenicity bioassay. During a 26-wk study to assess the potential carcinogenicity of oxymetholone using *p*-cresidine as a positive control, glass/polypropylene microchips (radio transponder identification devices) were subcutaneously implanted into male and female $p53^{+/-}$ mice. During week 15, the first palpable mass was clinically observed at an implant site. This rapidly growing mass virtually quadrupled in size by week 25. Microscopic examination of all implant sites revealed that 18 of 177 animals had a subcutaneous histologically malignant sarcoma. The neoplasms were characterized as undifferentiated sarcomas unrelated to drug treatment, as indicated by the relatively even distribution among dose groups, including controls. An unusual preneoplastic mesenchymal change characterized by the term "mesenchymal dysplasia" was present in most groups and was considered to be a prodromal change to sarcoma development. The tumors were observed to arise from dysplastic mesenchymal tissue that developed within the tissue capsule surrounding the transponder. The preneoplastic changes, including mesenchymal dysplasia, appeared to arise at the transponder's plastic anchoring barb and then progressed as a neoplasm to eventually surround the entire microchip. Capsule membrane endothelialization, inflammation, mesenchymal basophilia and dysplasia, and sarcoma were considered unequivocal preneoplastic/neoplastic responses to the transponder and were not related to treatment with either oxymetholone or *p*-cresidine.

Keywords. Transgenic mice; $p53^{+/-}$ mice; subcutaneous sarcoma; radio transmitter; solid state carcinogenesis; carcinogenesis models

INTRODUCTION

The emergence of transgenic technology has presented a more timely and novel approach to the identification of chemical carcinogens. Tennant et al (15) proposed a role for a battery of transgenic rodent models in the detection and classification of oncogenic materials. One of the suggested models, mice rendered deficient for 1 allele of the tumor suppressor gene *p53* (*p53* deficient, $p53^{def}$, or $p53^{+/-}$) [Donehower et al (3)] is currently under investigation for utility in identifying chemical carcinogens, and Boehringer Ingelheim Pharmaceuticals is taking part in this multifacility effort (13).

During a 26-wk study undertaken to assess the carcinogenic potentials of oxymetholone and *p*-cresidine in the $p53^{+/-}$ mouse, we employed subcutaneous implantation of BioMedic® radio transponders for the unique identification of animals. The BioMedic transponder is comprised of a small glass cylinder partially covered on 1 end by a larger polypropylene sleeve that contains a circular hole exposing the glass cylinder beneath. The ends of the plastic sleeve and glass cylinder are both convex. The glass surface regardless of location is generally uniform, smooth, and homogeneous, whereas the convex end of the polypropylene sleeve has the appearance of a twisted nipple (sprue). Extending from the sprue linearly along the side of the sleeve is a thin elevated seam, and at the open end of the sleeve, irregular and roughened barbs associated with the seams extend at an angle toward the glass end.

The BioMedic transponder has been used at Boehringer Ingelheim for nearly a decade. During our experience with nontransgenic rodents, we have routinely conducted both macro- and microscopic examinations of hundreds of implant sites. Whereas capsule formation around the transponder is usual, we have observed no evidence of carcinogenicity. Our experience with the transponders is consistent with that of earlier investigators, who reported no evidence of carcinogenicity after long-term implantation of transponders in mice and rats (1, 12).

Transgenic $p53^{+/-}$ mice are generally considered to be sensitive to genotoxicants but insensitive to nongenotoxins (15, 16). The glass and polypropylene components of the BioMedic transponder device are generally assumed to be devoid of mutagenic and/or cytotoxic components, so an observation of tumors, particularly as early as 26 wk, would not be predicted by this model. However, masses (undifferentiated sarcomas) were observed arising at the site of transponder implantation. Here, we describe our findings and suggest avenues of future research to enhance understanding of the utility of transgenic models in the detection and classification of chemical carcinogens.

MATERIALS AND METHODS

Animals and Animal Husbandry. Male and female transgenic mice, heterozygous for the *p53* tumor suppressor gene ($p53^{+/-}$ in C57Bl/6TacfBR mice) were purchased from Taconic Farms (Germantown, NY). Mice, approximately 6 wk of age and weighing 20–30 g upon

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receipt were acclimated to laboratory conditions for 8 days. Animals were individually housed in stainless steel wire-bottom cages and uniquely identified with IMI™ radio signal transponders (BioMedic Data Systems, Seaford, DE).

Transponders were subcutaneously implanted using a device that delivered the transponder subcutaneously into the interscapular region via a 12-ga needle. Aseptic techniques were followed.

Animal room conditions included 72°F ± 5°F at 50% ± 20% relative humidity, and a 12-hr on/off fluorescent light cycle. Animals had free access to pelleted rodent chow (PMI Feed No. 5002) and municipal water delivered via an automatic watering system. The animals were randomly assigned to experimental groups of 15 males and 15 females and were observed for evidence of morbidity and moribundity at least twice daily throughout the experimental period. Individual animal body weights were recorded weekly, and each animal was examined for the presence of palpable masses at the time of weighing.

Chemicals and Dosing. Oral gavage doses were administered 7 days/wk. Mice were administered 0.5% aqueous methylcellulose (10 ml/kg); 125, 625, or 1250 mg/kg of oxymetholone (CAS No. 434-07-1); or 200 or 400 mg/kg of *p*-cresidine (CAS No. 120-71-8). The vehicle for oxymetholone was 0.5% aqueous methylcellulose, and the vehicle for *p*-cresidine was corn oil. All animals received dose volumes of 10 ml/kg. Complete results of that carcinogenicity study will be reported elsewhere (Stoll et al, in preparation).

Necropsy, Tissue Collection, and Microscopic Examinations. Animals were subjected to a complete necropsy, and approximately 45 tissue samples, including samples from all gross lesions, were collected from all animals. Because we had not routinely collected samples from the transponder implantation site early on in the study and because the first transponder-associated masses were not observed until late in the study, the transponder implantation sites were not collected from animals that died early in the study. However, once the association of masses with the transponder site was made, we were able to collect samples from the transponder sites, with enclosed transponders, from 121 animals in the study. The tissue was preserved in 10% neutral buffered formalin and then trimmed and processed for light microscopic (hematoxylin and eosin [H&E] stain) and scanning electron microscopic evaluations. In addition to H&E staining, Masson's trichrome, periodic acid–Schiff, and factor VIII antibody detection techniques were employed.

DNA Isolation and Analysis of Heterozygosity. High-molecular-weight DNA was isolated from normal transponder sites and from transponder tumor tissue that was collected immediately at necropsy, flash frozen for not less than 2 min in liquid nitrogen, and stored at −80°C. Only tumors of sufficient size for dissection into approximately 50-mg pieces without compromising histology were collected. Loss of the wild type *p53* allele (chromosome 11) was determined as previously described (3, 4, 7). A 600-bp DNA probe spanning *p53* exons 2–6 was prepared (LR10 vector, courtesy of L. Donehower, Baylor College of Medicine, Houston, TX), randomly labeled with dCTP[$_a$^{32}P], and hybridized to *Bam*H1-restricted normal or tumor genomic DNA after electrophoresis and transfer to a nylon membrane. A radiographic image was prepared by exposing the hybridized filter to Kodak film for 48 hr at −80°C. Hybridization of the probe to the *p53* pseudogene (10 kb), null allele (6.5 kb), and wild type allele (5.5 kb) was observed. The mouse *p53* pseudogene (incorporation of a nonfunctional processed mRNA sequence) is located on chromosome 17 (17). Thus, the *Trp53-ps* signal represents diploid (2N or 2 chromosome 17 loci) DNA content, and the null allele is haploid (1N) and the wild type *p53* allele is haploid (1N) on each chromosome 11 for a *Trp53* 2N DNA content. Comparison of the signal intensity (from the Southern blot analysis) between the pseudogene and the wild type allele allowed detection of signal loss and was presumed to represent loss of heterozygosity or allele loss.

TABLE I.—The incidence of palpable masses at transponder implant sites in male (n = 15/group) and female (n = 15/group) *p53*$^{+/-}$ transgenic mice.

Group	Males	Females
Control	1	2
Oxymetholone		
Low dose	1	0
Mid dose	1	2
High dose	2	1
p-Cresidine		
Low dose	2	2
High dose	0	1

RESULTS

Survival

Eighteen of the 177 animals in the study were diagnosed with undifferentiated sarcoma arising at the transponder site. Of these 18 animals, 5 died prior to study termination. These were the animals that were the first to be identified with transponder site masses (102–151 days into the study), and they had the largest masses. Their deaths occurred between the eighteenth and twenty-fifth week of the study.

Macroscopic Examination of Transponder Sites

Of the 177 animals in the study, a total of 15 were observed to have detectable masses at the transponder site. Thirteen mice (6 males and 7 females) had palpable transponder site masses during the in-life phase of the study, and an additional 2 (1 male and 1 female) affected mice were identified at necropsy. These masses were not related to test substance administration; they were observed in controls as well as dosed animals (Table I). Observable masses were late developing but increased in size rapidly. For example, the first transponder site mass was identified during the fifteenth week; at that time it was 12 × 8 × 6 mm. By the twenty-fifth week, this mass reached a size of 55 × 39 × 35 mm, a 4-fold increase in size. Transponder site masses were generally firm and tan to tan-red in color.

TABLE II.—Incidences of transponder-associated proliferative and nonproliferative lesions in male and female *p53*$^{+/-}$ mice administered oxymetholone or *p*-cresidine for up to 6 mo and bearing subcutaneously implanted microchip radio transponder devices.[a]

Microscopic lesions at implantation site	Control		Oxymetholone						*p*-Cresidine			
			Low dose		Mid dose		High dose		Low dose		High dose	
	M	F	M	F	M	F	M	F	M	F	M	F
No. examined	13	12	12	8	12	8	13	9	14	6	9	5
Not remarkable	3	0	2	0	1	1	3	0	5	1	4	2
Nonproliferative lesions												
Mast cell infiltration	0	1 (8%)	0	0	1 (8%)	0	0	0	0	0	0	0
Perivascular mononuclear cell infiltration	0	1 (8%)	0	0	0	0	0	0	0	0	0	0
Pigmentation	0	3 (25%)	2 (17%)	0	1 (8%)	0	2 (15%)	0	0	0	0	0
Ulcerative dermatitis	1 (8%)	0	0	0	0	0	0	0	0	0	0	0
Subacute membrane inflammation	5 (39%)	5 (42%)	6 (50%)	4 (50%)	8 (67%)	3 (38%)	6 (46%)	5 (56%)	5 (36%)	0	4 (44%)	2 (40%)
Mesenchymal basophilia	2 (15%)	4 (33%)	2 (17%)	4 (50%)	0	3 (38%)	0	1 (11%)	0	1 (17%)	0	1 (20%)
Proliferative lesions												
Membrane endothelialization	8 (62%)	9 (75%)	9 (75%)	8 (100%)	9 (75%)	5 (63%)	7 (54%)	9 (100%)	6 (43%)	2 (33%)	4 (44%)	2 (40%)
Mesenchymal dysplasia	2 (15%)	1 (8%)	2 (17%)	5 (63%)	1 (8%)	1 (13%)	1 (8%)	0	0	0	2 (22%)	1 (20%)
Undifferentiated sarcoma	1 (8%)	2 (17%)	1 (8%)	0	2 (17%)	2 (25%)	2 (15%)	1 (11%)	2 (14%)	3 (50%)	0	2 (40%)

[a] Tissue from the transponder implantation site was not routinely collected. However, once identified as a common site for gross abnormalities, the transponder sites from 121 animals on study were examined.

FIG. 1.—Subcutaneous transponder sites in transgenic mice in unaffected stage and in various stages of tissue mass development. Panel 1: Transponder site cavity (Ts) prior to mass development. Transponder was located just beneath the skin (small arrow) and surrounded by a typical capsule membrane (large arrow) of condensed connective tissue in which mass (M) development begins. Orientation of the transponder capsule is glass extremity to the left and polypropylene extremity to the right. Panel 2: Mass development is apparent in association with the polypropylene extremity and was often observed to begin at the glass–polypropylene interface. At this stage, early mass development may or may not be macroscopically apparent. Panel 3: Fully developed mass predominantly at the polypropylene extremity. In later stages, the entire site may be involved.

Light Microscopy

Proliferative and nonproliferative changes observed at transponder sites are summarized in Table II. The low incidences of mast cell and mononuclear cell infiltration were considered incidental findings. The low incidence

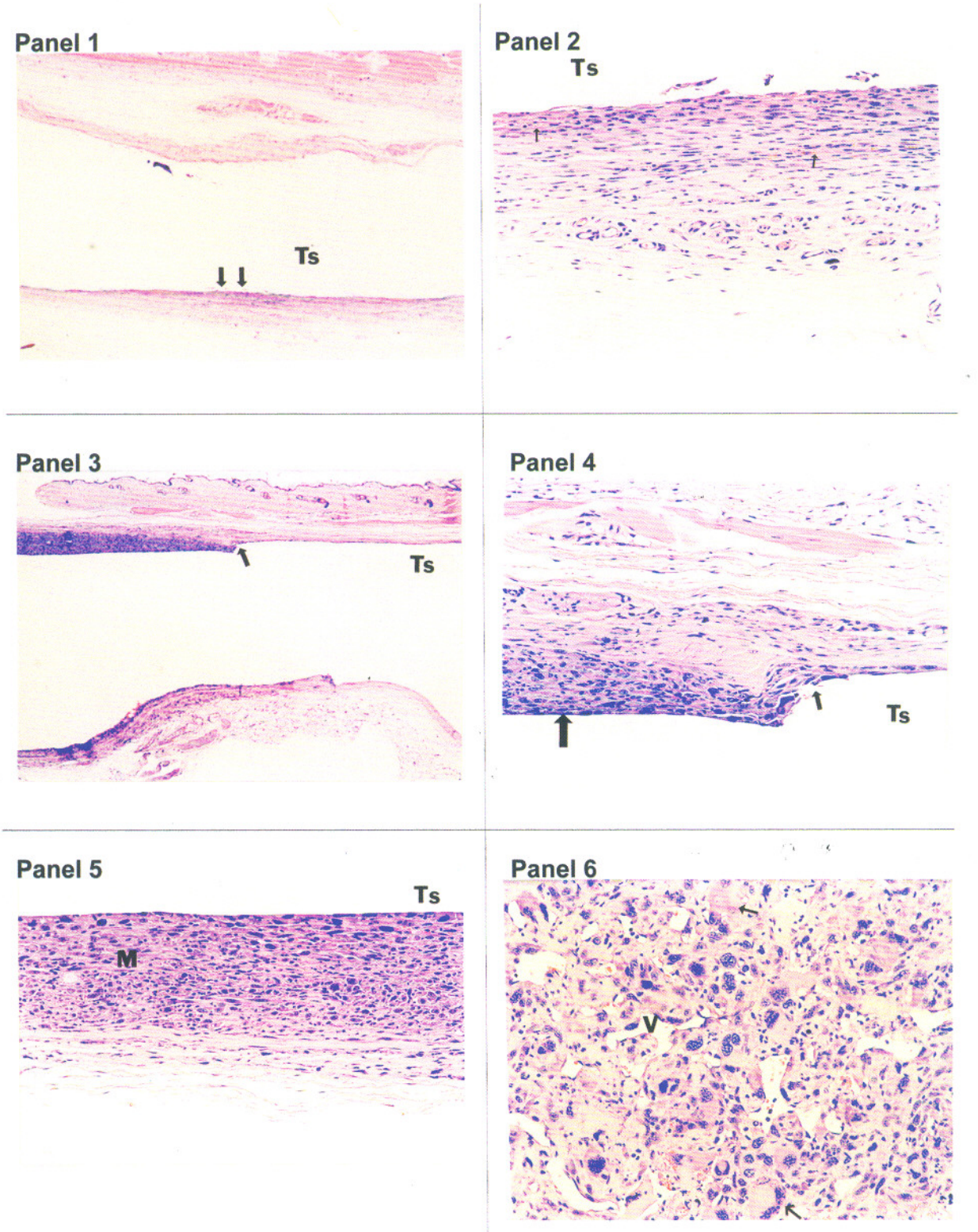

FIG. 2.—Development of mesenchymal dysplasia in a transgenic mouse, originating in the polypropylene end of the transponder tissue capsule with progression into undifferentiated mesenchymal neoplasia. Panel 1: Tissue capsule surrounding the transponder site cavity (Ts) with early mesenchymal dysplasia (arrows) originating in the polypropylene end. H&E. ×40. Panel 2: Higher magnification of the mesenchymal dysplasia indicated by arrows in Panel 1. Note the capsule membrane hypercellularity and the hyperchromatic spindle-shaped cells resembling fibroblasts adjacent to the transponder site cavity (Ts). Intramembranous lymphocytes and capillaries (arrows). H&E. ×200. Panel 3: Mesenchymal dysplasia originates at the barb (arrow) extremity of the polypropylene sheath, with progressive development of the mesenchymal dysplasia/sarcoma toward the polypropylene end of the transponder. H&E. ×40. Panel 4: Higher magnification of the area shown in Panel 3 at the barb showing transition

of pigmentation was likely due to the presence of the transponder, whereas membrane endothelialization, inflammation, mesenchymal basophilia, dysplasia, and sarcoma were considered unequivocal responses to the transponder.

The progressive development of transponder-associated lesions is shown in Figs. 1 and 2. Although there was variation in the extent of neoplastic involvement of tissue immediately surrounding the transponder site, it appeared that tumor(s) arose in the mesenchymal tissue surrounding the polypropylene component of the transponder, initially involving the barbed area and then in some cases extending completely around the entire transponder site. H&E staining revealed a tissue capsule formed immediately adjacent to the transponder and characterized by a broad dense eosinophilic layer of collagen with a few fibroblasts scattered within the collagenous matrix. Often a thin, flattened fibroblast-like lining layer on the inner surface of the tissue capsule was observed. Application of Masson's trichrome stain revealed that the capsule was intensely positive for mature collagen.

In animals with transponder site lesions, a variety of histologic changes were observed in the capsule surrounding the transponder (see Figs. 1 and 2). At the end of the plastic sleeve, near the centrally located tissue anchoring barbs, hyperchromatic dysplastic/neoplastic mesenchymal cells were observed within and beneath the dense layer of capsule collagen (Fig. 2, panels 1–6). These proliferative changes eventually extended outward into a large pleomorphic neoplastic mass located initially on the plastic end of the transponder (Fig. 1, panels 1–3). The mass itself consisted of densely packed pleomorphic, anaplastic, and hyperchromatic spindle-shaped, polygonal, or round cells having prominent cigar-shaped to round or bizarre large and/or multilobed vesiculated nuclei with 1 or more prominent nucleoli. In some tumors, multinucleated giant cells with nuclear lobation and variable to extensive amounts of cytoplasm were noted. The cytoplasm of some of these cells contained lipid vacuoles or had basophilic to esoinophilic abundant cytoplasm. The mitotic index for the tumor cells was generally moderate to high. Tumor cells were immediately surrounded with generally abundant collagen.

Neoplastic cells were arranged in a variety of patterns, including solid sheets, fascicles, and/or whorling patterns. The neoplasms had a variable vascular supply that was sometimes prominent, especially adjacent to the inner capsule. Vascularization usually consisted of mature capillaries and small veins, which sometimes formed cavernous networks. Throughout the neoplasm proper, small capillaries were often visible but appeared to be lined by nonneoplastic endothelium.

Areas of necrosis and hemorrhage were not uncommon, especially deep within the mass and sometimes near the periphery. Areas of hemorrhage and necrosis with pockets of degenerating neutrophils were noted in some areas of the tumors, and occasionally hemosiderin was noted, especially immediately adjacent to the inner capsule. Factor VIII antigen staining of tumor parenchyma for the identification of neoplastic vascular endothelial cells comprising the tumor mass or lining the capillaries and veins was inconclusive.

Electron Microscopy

Scanning electron micrographs of an unused transponder are shown in Fig. 3. The glass surface of the unit is smooth and homogeneous, whereas the edges of the polypropylene sheath, including the antimigration barbs, hole, and extrusion lines have jagged edges (Fig. 3, panels 1, 3, and 4). The surface of the polypropylene sheath (Fig. 3, panel 2) is scored with numerous extrusion lines, and the sprue has numerous jagged elevations.

Scanning electron micrographs in Figs. 4 and 5 depict subcutaneous tissues surrounding an excised transponder. Immediately surrounding the transponder, there was a band of dense homogeneous ground substance (capsule) with a superficial thin inner layer of large flattened polygonal cells with a fine fibrillar meshwork of cytoplasmic extensions of various lengths. On a longitudinal cut surface of tissue immediately adjacent to the capsule, there were stacked layers of densely fibrillar matrix with large polygonal to stellate cells with long fibrillar strands grouped in bundles surrounding them and sometimes extending from the cell bodies. In 1 section within the matrix adjacent to the capsule, there were distinct tubular structures that, when viewed on cut end, appeared to be vascular spaces with an occasional erythrocyte observed in the lumen. These spaces were not consistently noted in all areas of the mass, but in some areas they were prominent and abundant. The spaces themselves did not appear to be lined with abnormal cells. In many areas within the tumor mass itself, a dense meshwork of longitudinally oriented fibrillar strands formed interlacing bundles, with scattered elongated fibroblast-like cells interspersed. Extending into the spherical hole in the plastic were numerous pleomorphic polygonal cells with many surface microvillar projections embedded in a fibrillar meshwork. Some of these cells had a macrophage-like appearance, whereas others resembled fibroblasts.

Analysis of Genomic DNA

Southern blot analysis of genomic DNA is the most precise and efficient method for determining intragenic or intrachromosomal alterations and loss over extended genetic sequences due to the precise requirements for hybridization of complementary probe and wild type gene

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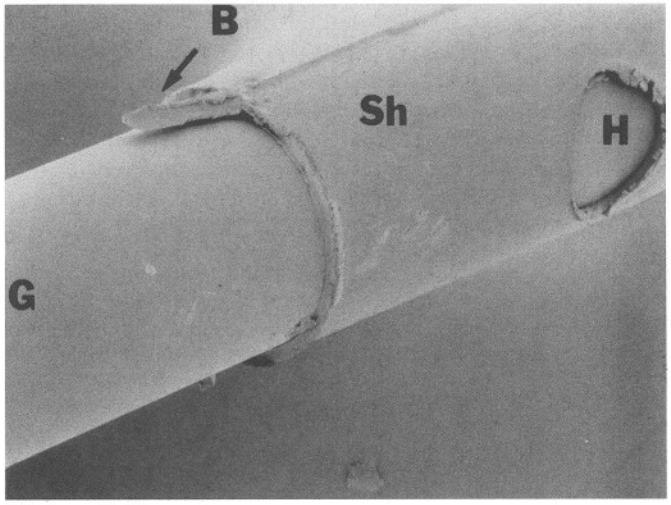
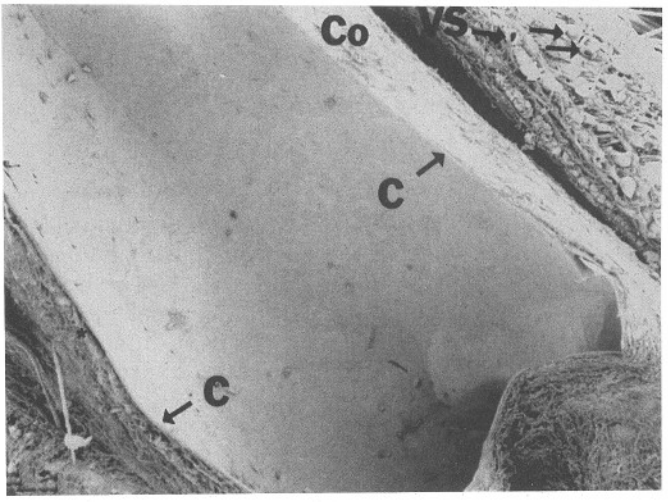
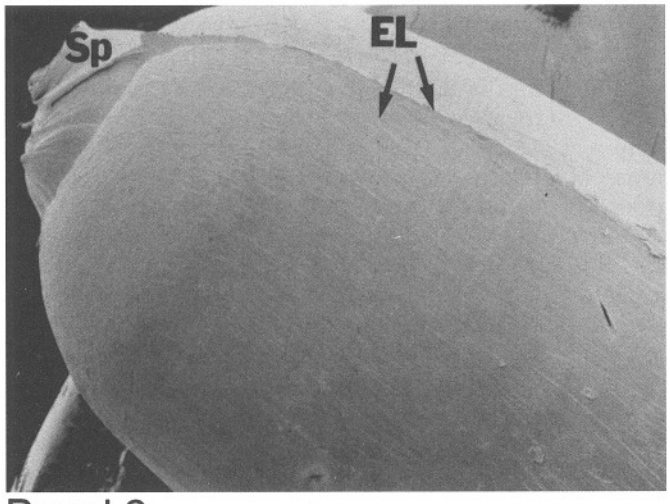
between dysplasia (small arrow) and neoplasia (large arrow). Note hyperchromasia and pleomorphism of the mesenchymal cells toward the polypropylene end. H&E. ×200. Panel 5: Early mesenchymal neoplasia (M) arising in the capsule surrounding the transponder site (Ts) on the polypropylene end. Cells demonstrate pleomorphism and atypia with hyperchromasia. At this stage, the lesion may not be macroscopically apparent. H&E. ×200. Panel 6: Undifferentiated mesenchymal sarcoma with giant cells (small arrows) with abundant acidophilic cytoplasm and 1 or more large bizarre nuclei and permeated by a vascular bed (V) lined with well-differentiated endothelial cells. H&E. ×200.



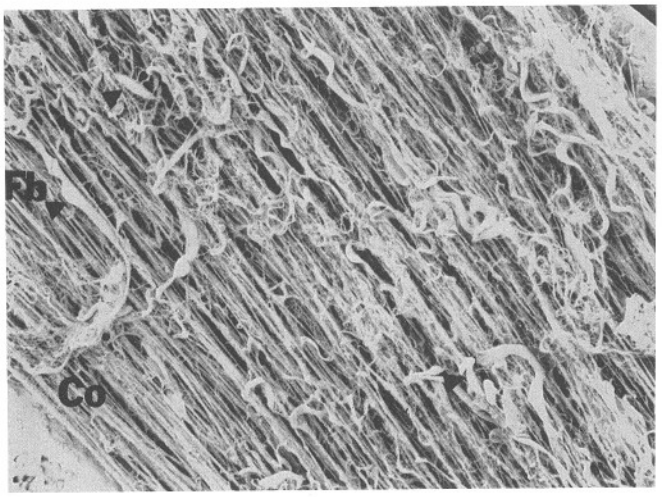
Panel 1



Panel 1



Panel 2



Panel 2

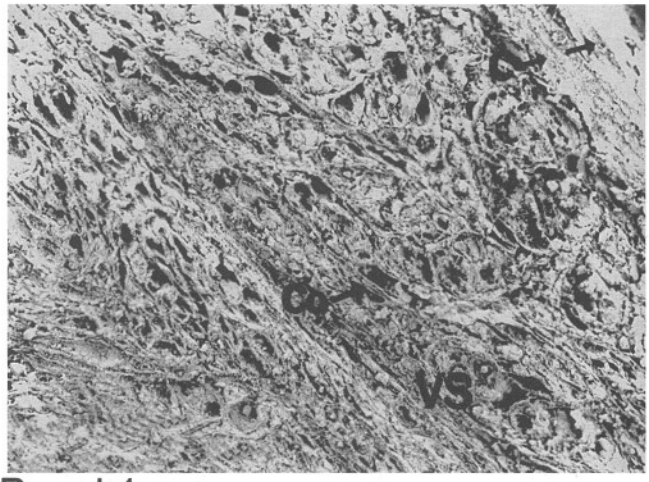
FIG. 3.—Scanning electron photomicrograph of transponder surface. Panel 1: Antimigration sheath (Sh) with barb (B) and hole (H). ×20. Panel 2: Surface of the polypropylene sheath with numerous extrusion lines (EL) and the sprue (Sp), which has the appearance of a twisted nipple with jagged elevations. ×40.

FIG. 4.—Scanning electron photomicrograph of tissue reaction to subcutaneous implantation of a transponder in a transgenic mouse. Panel 1: The space previously occupied by the transponder is surrounded by a dense, homogeneous fibrocollagenous capsule (C) adjacent to which is layered a meshwork of collagen bundles (Co), small vascular spaces (VS with arrows), and polygonal cells (asterisk). ×40. Panel 2: Numerous branching collagen bundles (Co) interspersed with fibroblasts (Fb) in the fibrocollagenous capsule. ×500.

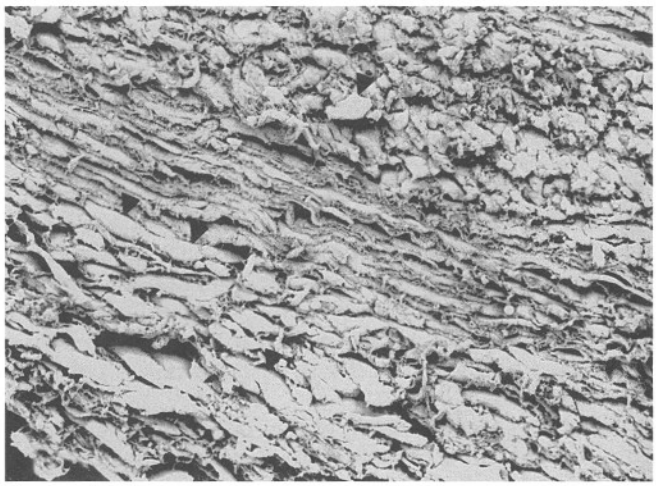
sequences. The *Trp53* loci in the C57BL/6 inbred mouse used in these studies are heterozygous because of the presence of the genetically altered nonfunctional *p53* null allele. Analysis of the tissue from masses arising at the site of a transponder (Fig. 6, lanes 1–7) showed losses of the wild type *p53* allele (5.5 kb) signal sequence (relative to the null allele, 6.5 kb), but no losses were detected in the *p53* null allele or the *p53* pseudogene (10 kb). Normal tissue specimens (liver) from mice with transponder inserts showed the expected heterozygous *p53* pattern, with 1 null allele and 1 wild type allele. Absence of null allele or wild type allele was demonstrated using normal tissues from untreated wild type (+/+) and nullizygous (−/−) mice, respectively.

DISCUSSION

The *p53* gene regulates the entry of DNA-damaged cells into specific stages of the cell cycle (6). Deletion of a single allele of this tumor suppressor gene in mice appears to be without effect on the development of spontaneous tumors, at least during the first year of life, but it imparts exquisite sensitivity to the mutational and carcinogenic effects of genotoxic chemicals. The oral (gavage) administration of genotoxic *p*-cresidine to $p53^{+/-}$ mice caused urinary bladder tumors within 26 wk (Stoll



Panel 1



Panel 2

FIG. 5.—Scanning electron photomicrograph of fibrocollagenous capsule and surrounding tissue mass in a transgenic mouse. Panel 1: Capsule (C) and sworls of interlacing bundles of pleomorphic polygonal cells accompanied by a delicate collagenous interstitial matrix (Co) with small vascular spaces (VS). ×700. Panel 2: The tissue mass adjacent to the fibrocollagenous capsule is characterized by layered sheets of polygonal to elongated flattened cells interspersed within a collagenous interstitial meshwork. ×500.

et al, in preparation). The National Cancer Institute (11) reported that *p*-cresidine caused urinary bladder tumors in both sexes of Fischer 344 rats and B6C3F$_1$ mice during a 2-yr bioassay. When we tested nongenotoxic oxymetholone in *p53*$^{+/-}$ mice, there was no evidence of carcinogenicity (Stoll et al, in preparation). The National Toxicology Program rat bioassay of oxymetholone is still in progress.

Because *p53*$^{+/-}$ mice are ostensibly insensitive to nongenotoxic proliferative agents, the observation of transponder implantation site sarcomas in 18/177 (10%) of the animals studied was surprising. However, in a recent publication (10), it was stated that "sarcomas may arise in *p53*$^{+/-}$ mice at subcutaneous sites of foreign body implantation" (Mahler et al, unpublished observations). Details supporting this statement were not presented. We have subsequently replicated this finding in 2 separate studies with the *p53*$^{+/-}$ mouse where transponder implantation site sarcomas were also observed. Because the component materials of the transponders are widely used in *in vitro* genotoxicity assays with no evidence of intrinsic mutagenic activity, these materials are generally assumed to be free of genotoxic potential. However, the possibility exists that leachates or even energy from the signal transmitted by the transponder are carcinogenic in *p53*$^{+/-}$ mice. These variables warrant further examination.

The carcinogenic potential of subcutaneously implanted transponders has been investigated in nontransgenic rodent strains. Rao and Edmondson (12) implanted microchips into male and female B6C3F$_1$ mice and conducted gross and microscopic examinations of implant sites after 3, 15, and 24 mo. Results of this study revealed nonneoplastic proliferative changes at the site of implantation as early as 3 mo after implantation. The smooth glass portion of the microchips was surrounded by a capsule composed of fibrocytes and collagen fibers. The polypropylene component was also surrounded by a similar capsule, and on occasion there was some evidence of inflammation. Device-associated neoplastic changes, however, were not observed in Rao's study, even in mice that carried the implanted transponder to the scheduled 24-mo study termination. Similar results were observed when microchips were implanted into male and female Sprague-Dawley (CD, VAF) rats for 52 wk with interim sacrifices and tissue examinations at 2, 12, 26, and 52 wk (1). The extent of the soft tissue reaction in the rat study was clearly time related, with immature fibrous connective tissue appearing in the subcutis 2 wk after implantation and with mature fibrous connective tissue surrounding the implant sites at later time periods. However, again there was no evidence of neoplastic change in animals that were observed for up to 1 yr. In other work, CD-1 mice that had received subcutaneous implants of the transponder also lacked a neoplastic response (Douglas J. Ball, personal communication).

The same identification transponders have also been used in the Tg.AC transgenic mouse. The Tg.AC mouse is another model being evaluated as a potential short-term alternative to the 2-yr rodent carcinogenicity bioassay (13). This transgenic mouse strain has the *v-Ha-ras* oncogene inserted into its genome, resulting in genetically initiated skin as a target for tumorigenesis (9). In a series of 26-wk studies between 1995 and the present, we have used the transponder in over 2,000 Tg.AC mice with no evidence of transponder-associated neoplasia. However, in an abstract recently presented by Johnson (5), subcutaneous sarcomas at the site of transponder implants were reported in approximately 1% of mice (strain not specified) used in chronic carcinogenicity studies. A more detailed and comprehensive description of these findings has not yet been published.

Nonneoplastic changes observed at the transponder implant sites of the *p53*$^{+/-}$ mice in our study were similar

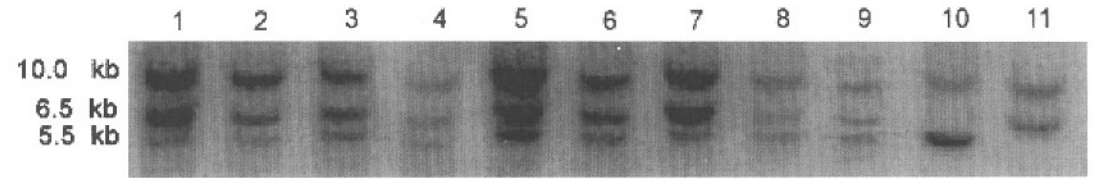


FIG. 6.—Electrophoretic separation of *Bam*H1-restricted genomic DNA from tissues from $p53^{+/+}$, $p53^{+/-}$, and $p53^{-/-}$ mice and identification of heterozygous *p53* alleles by Southern blot analysis. Lanes 1–7: Analysis of tissue masses from $p53^{+/-}$ mice, revealing loss of the wild type *p53* allele (5.5 kb) relative to either the null (6.5 kb) or pseudogene (10.0 kb) alleles. Lanes 8 and 9: Analysis of liver tissue from a $p53^{+/-}$ mouse that had implanted transponders, showing the expected distribution of pseudo, null, and wild type alleles in that tissue. Lane 10: Tissue from a $p53^{+/+}$ mouse showing the absence of the null allele and an unequivocal signal for the wild type allele and pseudogene. Lane 11: Tissue from a $p53^{-/-}$ mouse showing a strong signal for the null allele and pseudogene but no signal for the wild type allele.

to those observed in nontransgenic animals studied previously by others (1, 12). These changes included fibroblastic proliferation and collagen deposition, culminating in the formation of a protective membranous capsule around the transponder. The mechanism of the neoplastic proliferative response in the $p53^{+/-}$ mice remains obscure, but the response could be mediated by nonspecific mechanisms similar to the interference in cell communication proposed for solid state oncogenesis (2) or by foreign-body-induced production of cellular growth factors. Kuwashima et al (8) reported high levels of c-*fos* expression induced in murine sarcomas by foreign body (plastic) implants. DNA synthesis takes place during the proliferative response that culminates in formation of the protective subcutaneous capsule, and Brand et al (2) hypothesized that preneoplastic cells may be present in normal tissues and that implant-associated proliferation can provide conditions necessary for DNA synthesis to take place.

Induced expression of c-*fos* or other growth factors could mediate the proliferative changes observed in both transgenic and nontransgenic animals, whereas the *p53* phenotype ($p53^{+/+}$ or $p53^{+/-}$) influences the nature of the oncogenic response. Nontransgenic mice, homozygous for *p53*, may be relatively protected from the proliferation of pre-existing neoplastic cells, whereas *p53* haploinsufficiency may reduce the competence of this protective mechanism and render the animal uniquely sensitive.

Alternatively, the presence of the foreign body may elicit tissue reactions capable of generating genotoxic byproducts. Shi et al (14) recently hypothesized that crystalline silica-induced carcinogenesis in rat lung is secondary to oxidative stress mediated through reactive oxygen species (ROS) generated in response to the presence of the insoluble, nondegradable foreign substance. ROS have the potential to directly mutate DNA and *p53* and to promote c-*fos* expression and other growth factors.

Our Southern blot analysis of genomic DNA using a cDNA probe specific for the *p53* gene revealed an unequivocal transponder-associated loss of *p53,* but we are unaware of evidence that the transponder components have the potential to cause localized tissue reactions capable of generating genotoxic byproducts through the induction of ROS. Additional studies would be required to evaluate this possibility.

The components of the BioMedic transponder have been widely used in both standard human and experimental protocols, and there is little if any evidence that these materials possess carcinogenic potential for nontransgenic animals. Our results with $p53^{+/-}$ mice support the hypothesis that these animals represent highly sensitive and responsive models for the detection and characterization of the oncogenic potential of chemicals. The results of the present studies suggest that the $p53^{+/-}$ model may also have potential utility for the characterization of mechanisms of solid state carcinogenesis.

Also, although the incidence of transponder-associated sarcoma in this study was relatively high (18/177), there was an unequivocal association between the transponder and sarcoma that was unrelated to drug treatment. Therefore, interpretations of the drug-induced lesions were not affected. Nevertheless, it is important to make note of this transponder-induced lesion, especially when interpreting drug induced sarcoma such as that obtained with melphalan (10). Furthermore, the tumors observed in this study were classified as mesenchymal sarcomas and morphologically appear to be similar to the tumor described in a recent publication (10) as a sarcoma but not otherwise specified.

As is the case for all experimental models, blind leaps from the detection of tumors to the prediction of human health risk should be avoided. Carcinogenesis data in experimental species should never be used *in vacuo* for promulgation of regulatory policy. Our results with foreign body carcinogenesis in the *p53* transgenic mouse reinforces the importance of evaluating and integrating the sum total of understanding about both the experimental model and the test substance in question.

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