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Invited Review

Experimental models for carcinogenesis in the house musk shrew, *Suncus murinus*, Insectivora

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Summary. Animal carcinogenicity studies have mainly been performed on rodents. From the phylogenetic point of view, animals closer to humans must be included in these studies. Insectivora are considered to be the most primitive placental mammals and much closer to the early primates than rodents. Among the insectivora, the house musk shrew (*Suncus murinus*, family Soracidae), has been bred under laboratory condition. This animal is small having a short life span, and a comparatively low incidence of spontaneous tumor provides a useful animal model for tumor induction studies. We have examined the carcinogenicity of several chemicals known to produce tumors in rodents and found shrews, in general, to be sensitive to these chemicals but often showed different targets compared to rodents, and some chemicals tested were demonstrated not to be carcinogenic. Here we describe the carcinogenic studies performed in our laboratory and review other works including the occurrence of spontaneous tumors in shrews. Shrew carcinogenesis may fill up the gap of knowledge existing between the rodents and human beings.

Key words: Shrews, *Suncus murinus*, Insectivora, Carcinogenesis, Chemical carcinogens

Introduction

The genesis of cancer is associated with chemicals, viruses, radiation, as well as miscellaneous agents. Historically, chemical substances were the first to be identified to be the cause of human cancer. In 1755, Sir Percival Pott published a paper in relation with the increased incidence of cancer of the scrotal skin in chimney sweepers due to chronic exposure to tar and soot (Potter, 1963). While epidemiology can point to situations where a chemical is likely to be involved in the etiology of cancer, laboratory studies are necessary to identify the specific carcinogenic response. Yamagiwa and Ichikawa (1918) first demonstrated that skin tumors could be induced in rabbits by painting their skin with tar. Later in the 1930s, Kennaway and his colleagues (1955) fractioned chemically pure substances from tar and identified the carcinogenic effect in mice. Since then, hundreds of chemicals have been shown to be carcinogenic in animals. Animal carcinogenicity studies have usually been carried out on rodent systems, and have made a great contribution to the progress of cancer research. However, from the phylogenetic point of view, rodents are distant from humans, and they often metabolize chemicals in a way that differs from human metabolic pathways. Moreover, the high incidence of spontaneous tumors known in some strains may interfere with the interpretation. Therefore, other animal species are required for carcinogenesis experiment. Nonhuman primates have a closer phylogenetic relationship to man and may provide a more suitable model for humans (Adamson and Sieber, 1983). However, very long term studies are required due to their long life span. The tree shrew (*Tupaia glis*), a prosimian primate, has been used for chemical carcinogenic studies (Noyes, 1968; Adamson et al., 1970; Rao and Reddy, 1980), but as planned reproduction has not been achieved, a sufficient number of animals is hard to obtain.

Insectivora are considered to be the most primitive class of primates and also to be related to rodents (Romer and Parson, 1978). Among the insectivora, the house musk shrew (*Suncus murinus*), which belongs to the family of Soracidae, is widely distributed throughout the tropical regions of Asia and the Far East, and also inhabits the southern part of Japan. Shrews represent morphological and functional phenotypes similar to those of primates, and some may closely reflect features of human being. Shrews have been domesticated (Oda and Kondo, 1977) and planned reproduction has been achieved at the Central Institute for Experimental Animals, Kawasaki, Japan, and an outbred strain designated Jic:SUN maintained in a close colony was established (Matsuzaki et al., 1984, 1992). The sufficient numbers of this reproduction colony, the offspring of hybrids originally trapped in Okinawa and Nagasaki prefecture, Japan, and Jakarta, Indonesia, can be

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purchased from CLEA Japan, Inc., Osaka, when necessary. The shrew, as shown in Fig. 1, is a small body-sized animal (maximum body weight approximately 60 g in male and 40 g in female), with a short life span (< 2 years) and is useful for various types of experimental manipulations (Kondo et al., 1978; Furuyama et al., 1984; Matsuzaki et al., 1992). The advantages include the comparatively low incidence of spontaneous tumors, with the incidence of spontaneous tumors limited to pilosebaceous glands (Matsuyama and Suzuki, 1982). For these reasons, shrews are useful animals for chemical carcinogenicity studies. Organ specificity and spectrum of tumors may vary among different species. The results obtained in the insectivora using shrews as experimental animals is expected to fill the gap in the knowledge existing between the animal species of different order; e.g. between rodents and human beings. A variety of chemicals (Table 1) have been evaluated in this laboratory, and by others. Here we describe the chemical carcinogenicity study and report on the occurrence of spontaneous tumors.

Spontaneous development of pilosebaceous tumor

Matsuyama and Suzuki (1982) reported the spontaneous development of pilosebaceous tumors in the breeding colony captured in Nagasaki prefecture, Japan. Of the males over 51 weeks of age, 75% (12/16) developed multiple tumors located in dorsal skin. A single tumor mass contained various stages of transformation from adenoma to carcinoma. By contrast, only 1 out of 8 females aged 104 weeks developed solitary tumor. This sexual dimorphism indicates that male sex hormones play some role in the tumor development, which may be a model for pilosebaceous tumors in man.

1,2-Dimethylhydrazine (DMH)-induced musk gland tumors

Both male and female shrews possess a pair of side glands (musk glands) which consist of well-developed large elongated sebaceous glands, holocrine in nature. The exact function of the musk gland is still unknown. A high incidence of musk gland tumors was induced in female shrews by DMH (Tsubura et al., 1993a). DMH (Aldrich Chemical, Milwaukee, Wis) was dissolved in a 0.9% NaCl solution containing 1.5% EDTA and adjusted to pH 6.5 with 4% NaOH. The DMH solution was given weekly by subcutaneous (sc) injection in the back, beginning at 6 weeks of age, 14 and 28 times at the dose of 40 mg/kg and 20 mg/kg body weight, respectively, and the shrews were killed when tumors were visible or observed until 60 weeks of age. Musk gland tumors, usually bilateral, were induced in 82% (9/11) and 92% (12/13) of the shrews given 40 and 20 mg/kg body

Table 1. Chemical tested for carcinogenesis activity in shrews.

CHEMICALS	ABBREVIATION
1,2-Dimethylhydrazine	DMH
N-methyl-N'-nitro-N-nitrosoguanidine	MNNG
7,12-Dimethylbenz(α)anthracene	DMBA
Bytylated hydroxyanisole	BHA
Dimethylnitrosamine	DMN
N-methyl-N-nitrosourea	MNU

Table 2. Lectin-binding of normal musk gland and musk gland tumors.

LECTINS	PNA	WGA
Normal musk gland		
Duct	+	+
Acinus	+	-
Adenoma	+	-
Epithelioma	-	-

Fig. 1. An adult female house musk shrew (Jic:SUN strain).

weight, respectively, between 36 and 60 weeks of age, the mean age at harvest being 55.9 weeks and 53.1 weeks, respectively. Microscopically, they were either sebaceous adenoma (Fig. 2a) or sebaceous epithelioma (Fig. 2b). Human skin appendages and their tumors have characteristic lectin-binding (Tsubura et al., 1992a), and lectin histochemistry has been performed on normal musk glands (Aoki-Komori et al., 1993). Lectin-binding profiles for normal musk gland and DMH-induced tumors were examined histochemically on methacarn-fixed paraffin-embedded tissue specimens using biotinylated lectins (Vector Lab., Burlingame, CA), peanut agglutinin (PNA) and wheat germ agglutinin (WGA). In the normal musk gland, PNA labelled both the duct and the acinus, while WGA labelled the duct but not the acinus (Table 2). In adenomas, PNA remained positive but WGA was negative (Fig. 2c). In epitheliomas, WGA remained negative but PNA also lost its binding (Fig. 2d). This indicates that the tumors had originated from the acinar portion; adenomas showed similar lectin-binding to the normal musk glands, but loss of PNA-binding was characteristic in epitheliomas. Induced tumors were localized in the musk gland and no tumors were seen in the cutaneous sebaceous glands. Special localization of sebaceous tumors is known in rodents. The sebaceous tumors experimentally induced in rats by DMH and its metabolite azoxymethane (AOM), or by other chemicals, are usually localized in the sebaceous gland of the ear duct, called the Zymbal gland (Ward et al., 1973; Ward, 1974; Morii et al., 1979). In mice, tumors induced by DMH originate from the perianal or clitoral sebaceous

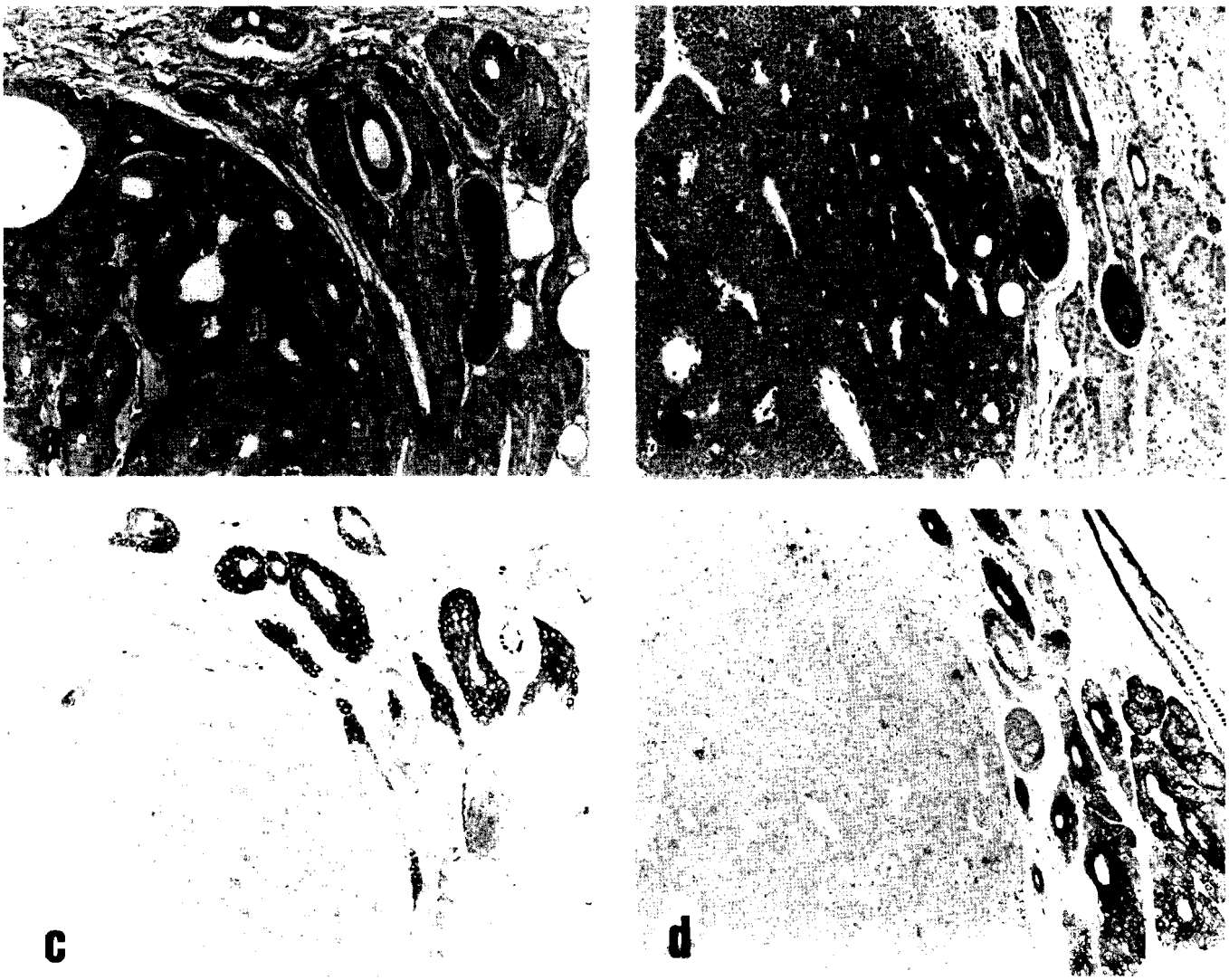

Fig. 2. Musk gland tumor. **a.** Sebaceous adenoma. H-E. **b.** Sebaceous epithelioma. H-E. **c.** WGA-binding is restricted to the ductal portion of the normal musk gland. Sebaceous adenoma lack the binding. WGA. **d.** PNA-binding is seen in both duct and acinus of the normal gland while sebaceous epithelioma is invariably negative. PNA. x 100

glands (Turusov, 1980). The musk gland tumor is androgen-dependent (Itami and Takayasu, 1983, 1984). Androgen-dependent tumors are rare. This may be a suitable model for studying the mechanism of androgen on tumor development. DMH, a colonotrophic carcinogen in rats (Ward, 1974), did not evoke colon cancers in shrews.

N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced esophageal carcinoma

The esophageal epithelium of rodents shows marked cornified changes which do not resemble those of the human esophagus, and they have a forestomach, which humans do not have. By contrast, the shrew esophagus is lined with stratified squamous non-cornified epithelium, and is devoid of forestomach. Therefore, the general appearance of the upper digestive tract of shrews much more resembles that in humans. In this respect, the shrew may represent a more suitable animal model for studying esophageal carcinogenesis. Epidemiological study suggests that nitroso compounds are an important etiological factor (Moses, 1991). Squamous cell carcinoma of the esophagus was selectively induced in female shrews by one of the nitroso compounds, MNNG (Tsubura et al., 1993b). MNNG (Nacalai Tesque, Kyoto) was dissolved in deionized water (1 mg/ml) as a stock solution and kept at 4 ºC in the dark. Working solutions (50 µg/ml), given to the animal as drinking water, were prepared every other day from the stock solution diluted with tap water just before feeding. Twenty female shrews were given MNNG beginning at 6 weeks of age for 30 weeks, then tap water until the end of the experiments (54 weeks of age). Animals were killed when they became moribund. Six shrews died 10-54 days after the MNNG administration began, but all 14 shrews survived until the end of MNNG administration and developed multiple esophageal lesions between 43 and 54 weeks of age (the mean surviving age was 48 weeks). Lesions were protruding, ulcerative, or superficial type of squamous cell carcinoma. Local invasion was seen but distant metastasis was not noted. Tumors were restricted to the esophagus and no neoplastic changes were found in other organs. Therefore, MNNG is a potent esophageal carcinogen in shrews. The spectrum of histological alteration of esophageal mucosa preceding carcinoma in animal models differs variously among species (Iizuka et al., 1982). In rats, induced esophageal carcinoma arises much more frequently via papilloma than via dysplasia (Pozharisski, 1990a). In humans, frequent association of invasive carcinoma with dysplasia and/or carcinoma in situ supports the hypothesis of dysplasia-carcinoma sequence (Takiyama, et al., 1992). Using the same induction method, sequential histological changes occurring in the shrew esophageal mucosa after MNNG treatment were examined (Fujita et al., 1994). As a result, basal cell hyperplasia was seen at 20 weeks of age, followed by dysplasia occurring at 25 weeks of age, which then progressed toward intraepithelial carcinoma to invasive squamous cell carcinoma at 35 weeks of age. Therefore, carcinoma development in shrews shows a stage of progression histologically comparable to that in humans.

The cell phenotype of MNNG-induced squamous cell carcinoma of the shrew was determined immunohistochemically using keratins as a probe (Takahashi et al., 1994c). Keratins are epithelial-specific intermediate-sized filament proteins, which are the products of various genes and are expressed in different cells and in cells at different stages of differentiation (Cooper et al., 1985). Moll et al. (1982) catalogued the major human keratins and identified them numerically as keratins 1-19. Biochemically, the characteristic keratin pattern in normal human esophageal epithelium is the presence of keratins 4-6, 13-17, and 19 (Moll et al., 1983; Grace et al., 1985). Keratins 4, 5 and 13 are seen in large proportions. In squamous cell carcinoma arising from the esophagus, keratin 13 is usually reduced (Moll et al., 1983), while the levels of keratins 14, 15 and 17 are increased (Grace et al., 1985). Immunohistochemically, the characteristic profile of esophageal squamous cell carcinoma was a strong and diffuse expression of keratin 14 and 16, strong but localized expression of keratin 17, and loss of keratin 13 expression (Malecha and Miettinen, 1991; Takahashi et al., 1995a). In shrew squamous cell carcinomas, although all cancer cells were keratin 13-positive, the expression was weak, and in contrast, diffuse and strong expression of keratin 14 was seen (Takahashi et al., 1994c). Therefore, both in humans and shrews, characteristic keratin expression showed the up-regulation of keratin 14 and the down regulation of keratin 13. Alterative bindings of lectins were seen during the course of shrew esophageal carcinogenesis (Takahashi et al.,, 1994a). In the normal shrew esophagus, soybean agglutinin (SBA), peanut agglutinin (PNA) and *Helix pomatia* agglutinin (HPA) binding was seen in suprabasal cells, and wheat germ agglutinin (WGA) and *Griffonia simplicifolia*-I agglutinin (GSA-I) to all layers of cells. In carcinomas, significant down-regulation of SBA, PNA, HPA and WGA binding was seen, while GSA-I binding was preserved.

7, 12-Dimethylbenz (α) anthracene (DMBA)-induced intestinal tumor

The intestine of shrew is characteristically short in proportions to the body length, where the large intestine, in particular, is extremely short (Kurohmaru et al., 1980; Kitamura et al., 1990; Kiso et al., 1991). Polycyclic aromatic hydrocarbons, originally derived from coal tar, are among the most extensively studied carcinogens. Among them, DMBA is one of the most powerful chemical carcinogens that has a varied range of target organs in rodents (Huggins, 1979). Male and female shrews treated with various doses of DMBA administered by either gastric intubation (per os) or

intraperitoneally (ip) developed intestinal tumors (Tsubura et al., 1992b). Shrews received gastric intubation of 10 mg DMBA (Eastman Chemical, Rochester, NY) dissolved in sesame oil at 50 days of age, or received 2 x 10 mg doses at 50 and 55 days of age, or received weekly ip administration of 1.25 mg or 2.5 mg DMBA emulsion (Upjohn, Kalamazoo, MI) for 4 weeks or for 8 weeks from 8 weeks of age. Regardless of route or dose of DMBA administration, intestinal tumors developed in up to 33-83% of the shrews. The induced tumors, which began to appear by 15 weeks after the initial treatment, were randomly distributed throughout the intestine, and were adenocarcinomas similar to those found in humans. A different route of administration produces a specific type of tumor. Leukemia developed when DMBA was administered ip in a dose-dependent manner (≥ 10 mg) which reduced the survival rate. On the other hand, per os route of either 10 or 20 mg of DMBA did not evoke leukemia. Various chemicals are known to induce intestinal tumors in rodents, but DMBA has not been reported to cause such tumors (Pozharisski, 1990b).

7, 12-Dimethylbenz (α) anthracene (DMBA)-induced leukemia

As already mentioned, leukemia is evoked by repeated ip injection of DMBA (Tsubura et al., 1992b). DMBA emulsion (Upjohn, Kalamazoo, MI) was administered ip at a dose of 1.25 or 2.5 mg once a week, with either four or eight doses being given from 8 weeks of age and observed until 50 weeks of age (Tsubura et al., 1991b). Leukemia, lymphatic and/or mast cell type developed in a dose-dependent manner. Incidence and the mean latency was 100% (9/9); 15.1±3.3 weeks in 20 mg (8 x 2.5 mg) group, 50% (5/10); 15.0±5.2 weeks in 10 mg (8 x 1.25 mg) group, 56% (5/9); 23.6±14.6 weeks in 10 mg (4 x 2.5 mg) group, and 0% (0/10) in 5 mg (4 x 1.25 mg) group. Massive splenomegaly was generally seen (1888±1489 mg in leukemic shrew vs 418±133 mg in controls; $p<0.01$), which was occasionally accompanied by infarction, necrosis and haemorrhage. Leukemic cell invasion, regardless of cell type, was always seen in the spleen, while other organs (liver, bone marrow, lymph nodes and thymus) were not always involved. The major evidence of leukemic involvement found in the spleen suggests that the spleen is a favourable locus for the generation of leukemic cells. A characteristic feature of shrew spleen is that it exhibits considerable haemopoietic activity throughout adult life (Fukuta et al., 1982). It is known that DMBA induced several types of leukemia in rodents (Huggins, 1979), but there is no report of mast cell leukemia.

Butylated hydroxyanisole (BHA)-induced adenomatous hyperplasia and adenomas of the lungs

Amo et al. (1990) reported that chronic feeding of BHA induced adenomatous hyperplasia and adenomas of the lungs in shrews. BHA, widely used as a preservative in food, is known to induce tumors of the forestomach in rats, hamsters and in mice (Ito et al., 1983; Masui et al., 1986). In rodents, BHA does not show a tumorigenic potency in lungs. BHA (Wako Pure Chemical Industries, Osaka) mixed with the basal diet at the concentration of 2.0, 1.0, 0.5 and 0% was fed to male and female shrews from 5 weeks of age for 80 weeks. All of the shrews given the 2.0% BHA diet died of bleeding in the gastrointestinal tract within 8 weeks after the treatment. The majority of the shrews in other groups survived for more than 52 weeks. In both sexes in the 1.0 and 0.5% groups, adenomatous hyperplasia of the lungs, and multiple small lesions scattered in the whole lobes, were detected in 59-67% of the shrews, while those in the 1.0% group had many more foci than those in the 0.5% group. In adenomatous hyperplasia, proliferation of bronchiolar epithelial cells extended onto the alveolar septa, and electron microscopy showed Clara cell origin. In addition, adenomas consisting of atypical cells with larger nuclei and basophilic cytoplasm were seen in a few shrews. In three shrews (*Tupaia glis*), a prosimian primate, 2-2'-dihydroxy-di-n-propylnitrosamine (DHPN) induces similar pulmonary lesions of Clara cell origin (Rao and Reddy, 1980). The histological architecture of the lung of the shrew is the same as that of humans.

7,12-Dimethylbenz (α) anthracene (DMBA)-induced sarcoma

After administration of 1 mg of crystalline DMBA (Eastman Chemical, Rochester, NY) sc to 4-week-old female shrews which were observed until 50 weeks of age, sarcomas were induced in 69% (9/13) at the mean of 41.4±5.8 weeks after the treatment (Tsubura et al.,

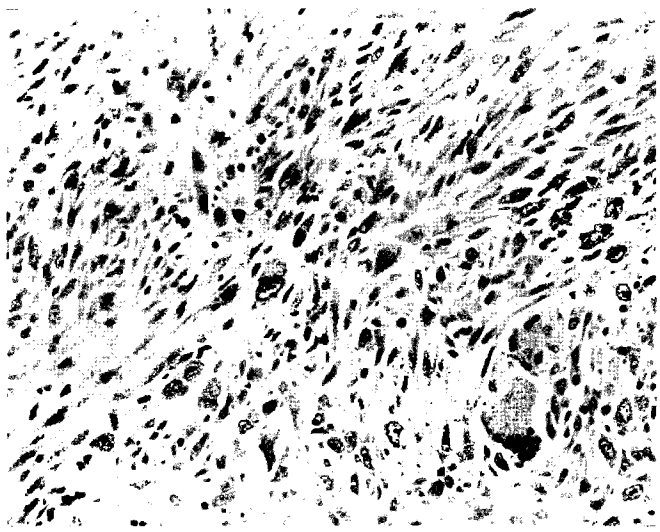

Fig. 3. Fibrosarcoma. Tumor is composed mainly of spindle cells and bizarre giant cells are also seen. H-E x 200

1991b). Tumors were induced at the carcinogen inserted site and no metastasis was found. Tumors were composed mainly of spindle cells, and in some areas, bizarre giant cells were admixed (Fig. 3). Myxoid change or granular degeneration was also observed. Invasion was seen, but no distant metastasis was noted. In rodents, sarcoma is induced locally by polycyclic aromatic hydrocarbons (Huggins, 1979). 3-Methylchoranthrene and 3,4-benzypyrene administered to tree shrews (*Tupaia glis*) also develop sarcomas (Noyes, 1968; Adamson et al., 1970). Therefore, fibroblast of the rodents, prosimian primates as well as shrews are universally susceptible to polycyclic aromatic hydrocarbons.

Other chemicals tested

Nitrosamines are suspected to play a role in human esophageal cancer (Yang, 1980). They are known to induce gastrointestinal cancer and possibly other cancers in experimental animals (Magee and Barnes, 1967). Dimethylnitrosamine (DMN) produces liver and kidney tumors in rodents (Hard, 1979); it is not carcinogenic in primates, but reveals severe hepatotoxicity (toxic hepatitis, cirrhosis, and hyperplastic nodules) (Adamson and Sieber, 1983). The tumor induction in shrews has not been attempted. Continuous administration of DMN (Nacalai Tesque, Kyoto) in the drinking water at a concentration of 5 or 15 ppm was started at 8 weeks of age. In the 5 ppm group, all 9 female shrews died at 8-16 weeks (mean 10.8 weeks), and in the 15 ppm group, both female shrews died 2-4 weeks (mean 3 weeks) after the treatment began. DMN was diluted in physiological saline and was given 30 mg/kg body weight to female shrews at 8 weeks of age, or given 10 mg/kg body weight twice at 8 and 9 weeks of age. In the 30mg/kg group, all shrews died within 2 days post-treatment due to liver damage, mimicking human fulminant hepatitis. Similar liver injury has been reported in rats injected at the same dose level (Pritchard and Butler, 1989). In contrast, no acute toxicity occurred in the low dose (10 mg/kg x 2) group, and tumor development was not seen until they were 50 weeks of age (10 shrews). The sensibility of shrew liver to chemicals differs from that of rodents (Lin et al., 1986). Tumor induction in shrews by DMN has not been achieved.

N-methyl-N-nitrosourea (MNU), a direct-acting carcinogen, is a potent carcinogenic compound. In rodents, the site of carcinogenic action of MNU varies with time, dose and route of administration (Narisawa et al., 1976; Anisimov, 1988; Thompson et al., 1992). In addition to its varied carcinogenic activity in rodents, MNU induces tumors in the upper digestive tracts in nonhuman primates (Adamson et al., 1977). MNU (Nacalai Tesque, Kyoto) was dissolved in 0.9% NaCl solution containing 0.05% acetic acid (Takahashi et al., 1995b). At 35 days of age, the ip injection of 50, 25, 10 and 5 mg/kg MNU solution was made along the ventral midline of the shrew abdomen. In the 50 mg/kg group (5 shrews), one shrew died on day 1 and the other 4 on day 2. In the 25 mg/kg group (5 shrews), three shrews died on day 4 and the other 2 on day 5 after MNU injection. In the 10 and 5 mg/kg groups (15 shrews each), acute toxicity was not found, and for observation until 50 weeks of age, a high occurrence of tumor was not seen. However, a few intestinal tumors mainly restricted to the large intestine were found in shrews over 44 weeks of age although we cannot conclude that they were due to MNU (Fig. 4). Intrarectal administration of MNU produces large intestinal carcinomas in mice, rats, and guinea pigs (Narisawa et al., 1975, 1976). This may be the desirable route for large intestinal carcinogenesis in shrews by MNU.

Spontaneous mammary tumor and mammary tumor virus

Shrews possess 3 pairs of mammary glands at the inguinal region (Yokoyama et al., 1980). The mammary gland is composed of luminal cells and basal (myoepithelial) cells (Tsubura et al., 1991a), but the virgin female has only a rudimentary duct system (Kitoh et al., 1985). The hormonal control of the shrew mammary gland is not yet well known; the action of the prolactin-releasing drug differs from that of the rodents (Takahashi et al., 1994b). In our experience of handling virgin female Jic:SUN strain, we have never seen spontaneous mammary carcinomas nor succeeded in inducing mammary tumors by chemicals. However, mammary carcinomas sometimes occur spontaneously in shrews (7%; 2/29) (Amo et al., 1990). A culture cell line (Sm-MT) was established from one of the spontaneously-occurring papillotubular adenocarcinoma developed in a two-year old shrew belonging to a line trapped in southwestern Japan. Interestingly, Tsutsui et al. (1985) reported that this Sm-MT produced a new type

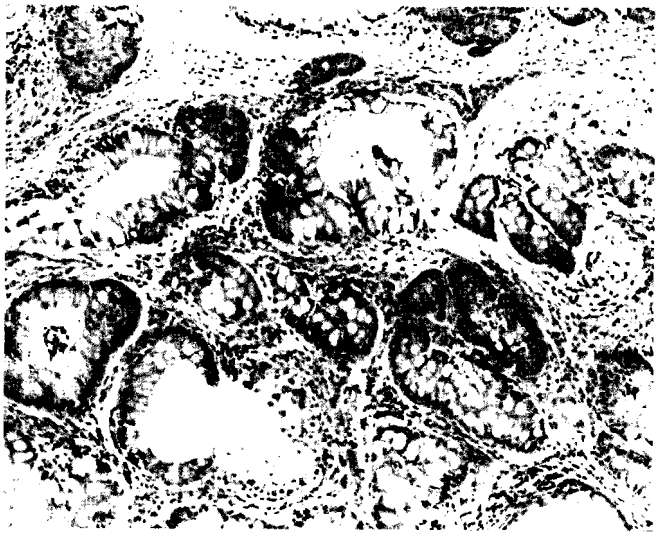

Fig. 4. Mucin-secreting carcinoma. This tumor originated from the large intestine. H-E. x 100

of retrovirus (Sm-MTV). Type C retrovirus is found in almost all vertebrates in association with leukemia and sarcoma (Bernhard, 1960). In contrast, mammary tumor-associated retroviruses, type B mouse mammary tumor virus (MMTV) (Bentvelzen and Hilgers, 1980) and type D Mason Pfizer monkey virus (MPMV) (Chopra and Mason, 1970), is restricted to mice and monkeys, respectively. Both the type B and type D virus particles are characterized by the presence of intracellular precursor particles (intracytoplasmic A particles), as well as the presence of reverse transcriptase with Mg^{2+} preference. Sm-MTV possesses constitutive Mg^{2+}-dependent reverse transcriptase (Tsutsui et al., 1985). The extracellular mature virion with a spikeless envelope with a centrally located nucleoid, and precursor of the mature virion with doughnut-shaped and horse-shaped intracellular A particle is morphologically different from MMTV and MPMV (Malavasi Yamashiro et al., 1986). The discovery of Sm-MTV is of importance in the phylogeny of retroviruses.

Conclusions and perspective

The actions of chemical carcinogens as well as spontaneous tumors in the house musk shrew, *Suncus murinus*, a new laboratory animal established in Japan, have been reviewed. In general, shrews were sensitive to tumor induction by chemical carcinogens, but some chemicals (e.g. DMN and MNU) clearly associated with rodent tumors were not readily demonstrated to be carcinogenic in shrews. Compared to widely-used rodent models, the chemicals tested had different actions and different targets but distinct organotropism in shrews. DMH, MNNG, DMBA and BHA showed distinct organ-specific carcinogenicity in shrews. On the other hand, unlike the rodents, colon cancer was not induced by DMH (Ward, 1974), gastric carcinoma was not evoked by MNNG (Ohgaki et al., 1991) and mammary carcinoma was not produced by DMBA or MNU (Huggins, 1979; Thompson et al., 1992; Takahashi et al., 1995b). Carcinogenic potential in animal does not necessarily mean that the chemical is a human carcinogen. However, the demonstration of carcinogenicity and the determination of the target organ in species phylogenetically closer to humans than the rodents will provide valuable information.

Planned production of shrews has been established, and sufficient numbers of animals (Jic:SUN strain) can be purchased from the breeder (CLEA Japan Inc.). We are keeping shrews at room temperature maintained in 22±2 °C and relative humidity 60±10% with a 12-h dark-12-h light cycle, housing 3-5 animals in a plastic cage with sterilized whit pine chips as bedding, and feeding a special pellet diet for shrews (CIEA-305; CLEA Japan, Inc.) and water freely. The appropriate percentage composition of the CIEA-305 is: moisture 4.7%, crude protein 38.4%, crude fat 6.4%, crude fibre 1.7%, crude ash 6.9%, and nitrogen-free extract 41.9% (Matsuzaki et al., 1992). In our experience, they are easy to handle and are resistant to infection. However, when housing more than 2 shrews per cage, caution must be paid to moribund shrews because of cannibalism. From a comparative point of view, the findings presented here provide important basic information. Together with the murine model which has been used extensively as experimental animal for the elucidation of the etiology and pathogenesis of human tumors, further studies in shrews may offer more opportunity for a better understanding of human tumors.

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