highly metastatic ductal carcinomas (17). Expression of *p16-Ink4a*, a Ras inducible gene (59), was clearly elevated in Hras250 rat pancreatic tumor tissue, indicating that transcription of *p16-Ink4a* can still be activated in the tumor cells. In agreement with the finding that the tumor tissue had more PCNA positive cells than the surrounding tissue, *cyclin D1* expression was also induced. These results suggest that the cells comprising the pancreatic adenocarcinomas are actively proliferating, but that the Rb pathway, which plays an important role in coordinating movement through the cell cycle, is probably intact.

Finally, expression of both *cyclin D2* (*Ccnd2*) and *cyclin D3* (*Ccnd3*) was reduced compared with control pancreatic tissue. This is consistent with a clonal origin of the tumors.

Table I. Summary of cellular characteristics

	Early lesions			Advanced lesions
	Intraductal epithelium		Ductular lesion	Duct epithelia lesion
	PanIN-1	PanIN-2/3		
β-Catenin	+	+	+	+
CK19	+	+	+	+
CK7	+	+	+	+
EGF	±	±	±	+
EGFR	±	+	±	+
MMP-7	–	±	±	+
COX2	+	+	±	+
PAS	–	– to ±	±	±
Alcian blue	–	±	±	±
Amylase	–	–	–	–
Chymotrypsin	–	–	–	–

+, positive; ±, weakly or partially positive; –, negative.

Taken together, Hras250 rat pancreatic carcinogenesis is initiated by expression of the Ha-*ras*^{G12V} oncogene. Proliferation is further stimulated by an EGF–EGFR autocrine loop and decreased expression of inhibitory proteins such as *Madh4* and *p21-Cip1/Waf1*. The resultant increase in proliferative capacity of the cells is demonstrated at the cellular level by increased expression of *cyclin D1* and PCNA and at the tissue level by tumor formation. However, while the cells comprising the tumors exhibit abnormally high proliferation, p53 and Rb signaling pathways appear to be intact, suggesting essentially normal movement through each cell cycle.

Summary

Few animal models exist that recapitulate human pancreatic ductal carcinogenesis. In this report, we document the establishment of a rat line, Hras250, carrying a human Ha-*ras*^{G12V} oncogene under the control of the *Cre/lox* system. Injection of *Cre*-carrying adenovirus induces tubular adenocarcinomas of ductal and ductular origin in the pancreas with high penetrance and short latency. These adenocarcinomas arise from centroacinar cells, intercalated ducts and duct epithelium, but not acinar cells (Figure 8). Also, a preliminary survey suggests that Ha-ras^{G12V}–initiated carcinogenesis may occur through proliferative mechanisms without the disruption of the p53 or Rb signaling pathways. These results, taken in conjunction with the morphology of the lesions, suggest that this animal model exhibits characteristics of premetastatic pancreatic carcinomas that are progressing towards frank malignancy. The Hras250 rat carrying a Cre recombinase-regulated human Ha-*ras*^{G12V} gene promises to be an excellent tool for the analysis of pancreatic tumor histogenesis, screening and therapeutics.

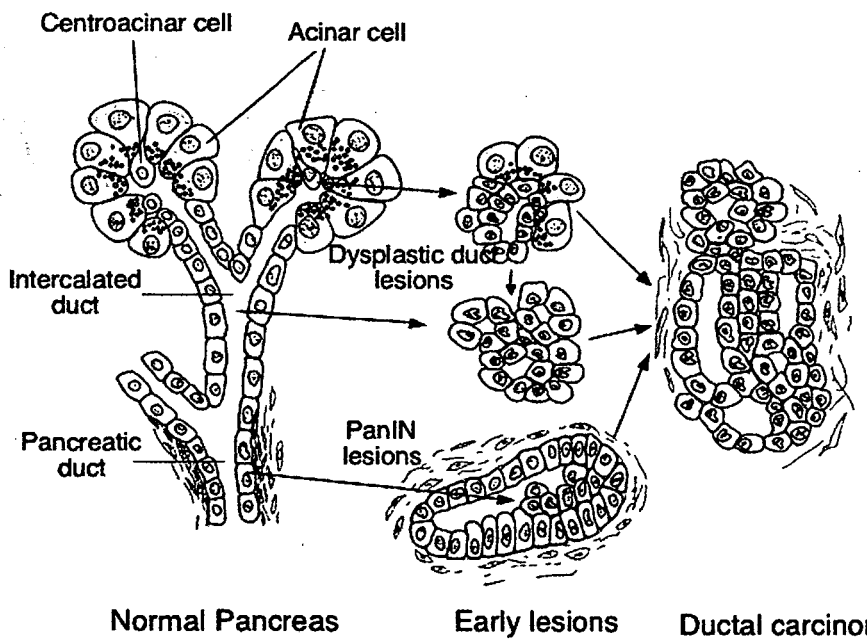

Fig. 8. Schematic presentation of cytogenesis of pancreatic ductal adenocarcinomas induced in human Ha- *ras*^{G12V} transgenic rats. Precursor lesions are hyperplastic and dysplastic proliferation of centroacinar cells, intercalated ducts and duct epithelium (PanIN-like lesions), but not of acinar cells. Irrespective of cytogenesis, the eventual morphology is adenocarcinoma with ductular phenotype.

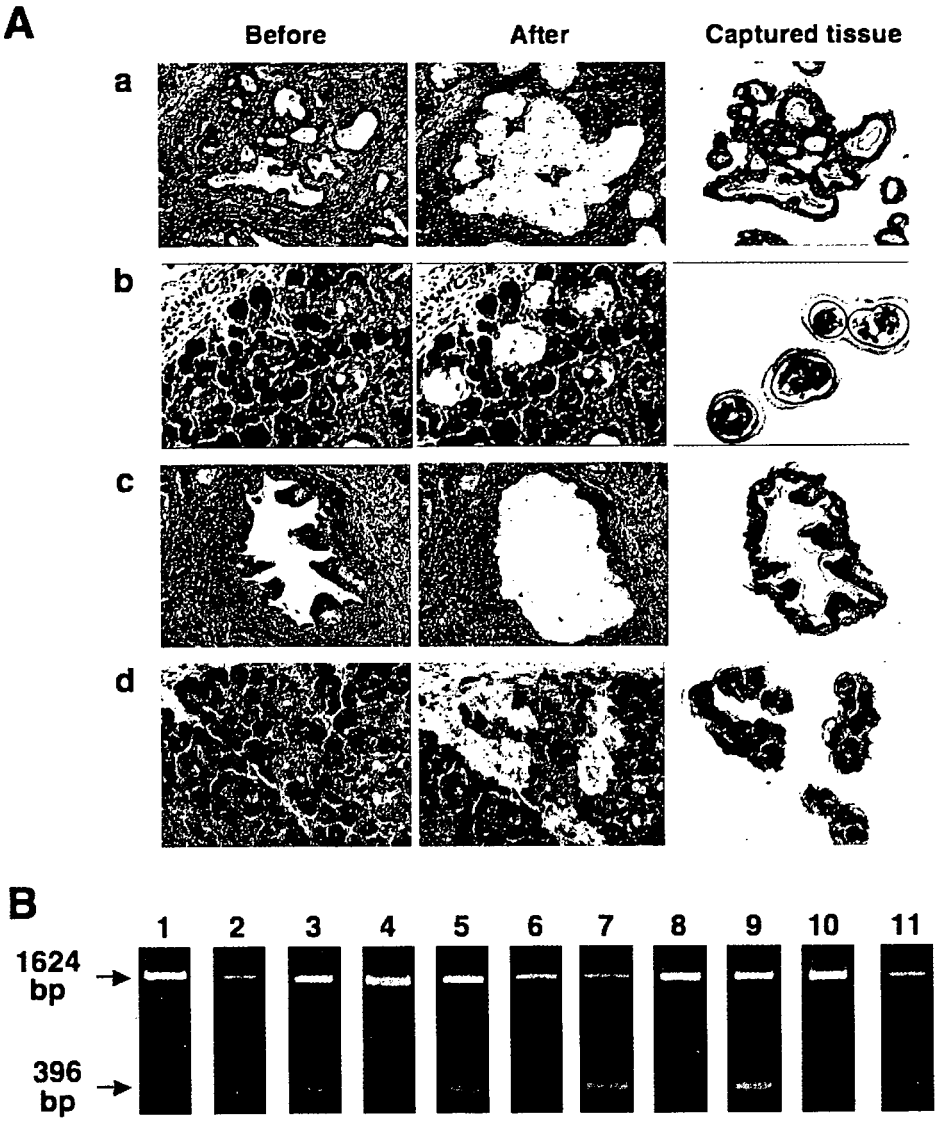

Fig. 9. Detection of recombination of the transgene by LCM. (**A**) Tissue from a pancreas of an AxCANCre-treated animal captured by LCM. Panel a, adenocarcinoma; panel b, mixed population of proliferating centroacinar cells and intercalated ducts; panel c, PanIN-2/3-like lesion; panel d, normal looking acinar cells surrounding proliferating centroacinar cells and intercalated ducts. (**B**) PCR amplification of the transgene. The 1624-bp band corresponds to the unmodified transgene, and the 396-bp band corresponds to transgenes that have undergone recombination. 1, acinar tissue from an AxCAwt-treated pancreas; 2, duct from an AxCAwt-treated pancreas; 3 and 4, captured tumor tissue from panel A-a; 5 and 6, mixed population of proliferating centroacinar cells and intercalated ducts from panel A-b; 7 and 8, PanIN-2/3-like lesion from panel A-c; 9,10 and 11, normal looking acinar cells surrounding a mixed population of proliferating centroacinar cells and intercalated ducts from panel A-d.

The ease of tumor induction; the similarity to human pancreatic tumors; the high penetrance, rapidity and multiplicity of tumor induction; and the large size of the rat pancreas are all factors that will facilitate research into activated *ras*-associated pancreatic neoplasia using this animal model.

Supplementary material

Supplementary data are available at *carcinogenesis* online.

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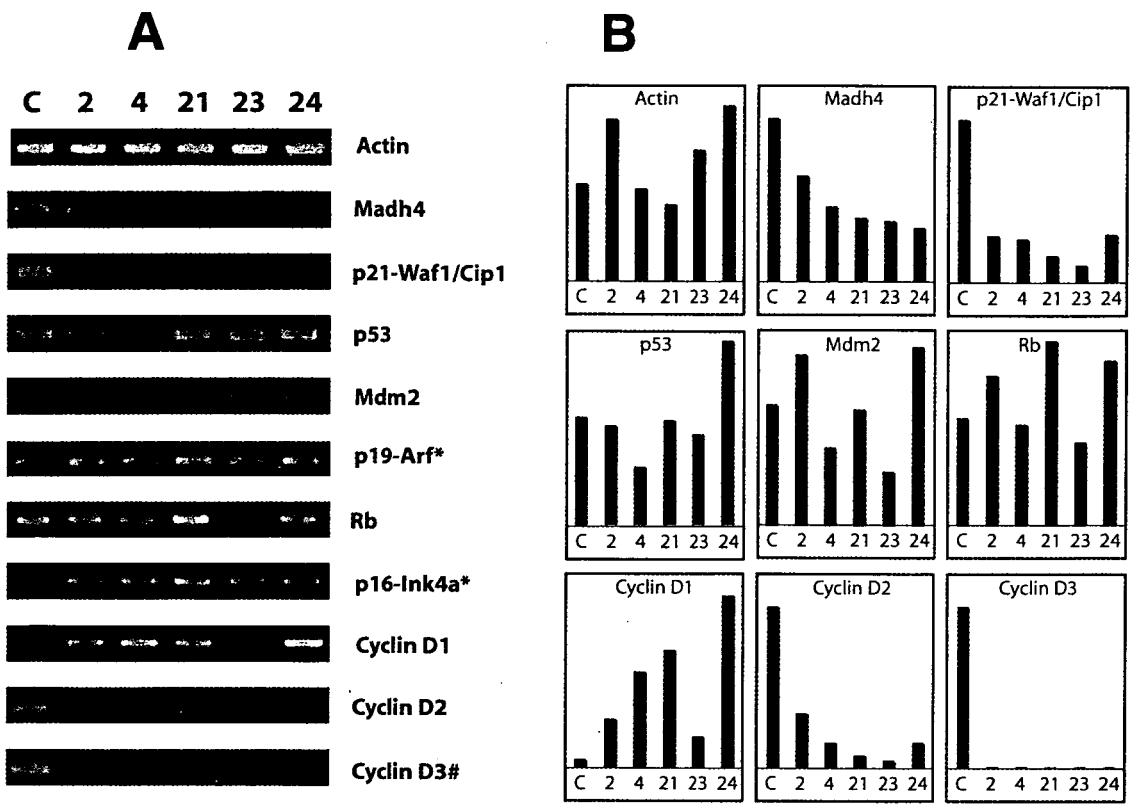

Fig. 10. RT–PCR analysis of pancreatic tumors in Hras250 animals. Total RNA isolated from an AxCAwt-injected Hras250 rat pancreas (control) and five pancreatic tumor samples from AxCANCre-injected Hras250 rats were used to generate cDNA by reverse transcription. The cDNA was amplified using primers specific for *Madh4, p21-waf1/cip1, p53, Mdm2, p19-Arf, Rb, p16-Ink4a* and *cyclin D1, D2* and *D3*. Amplification of β-actin served as an internal control. (**A**) PCR was stopped during the log-linear phase of the reaction and amplicons were run out on an agarose gel. (**B**) Graphical results of semi-quantitative real-time PCR analysis. (Numerical data can be found in Supplemental material.) C, Control pancreas; 2, 4, 21, 23 and 24, pancreatic tumors from AxCANCre-treated Hras250 animals. *Amplicons were not generated for either p19-Arf or p16-Ink4a during real-time PCR. Therefore, nested PCR was used to generate amplicons for these cDNAs. Quantification was not attempted for the nested PCR products. # Amplicons were generated for cyclin D3 during real-time PCR only for the control tissue and not for any of the tumor tissues.

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Possible application of human c-Ha-ras proto-oncogene transgenic rats in a medium-term bioassay model for carcinogens

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Running title: Hras rat medium-term carcinogen bioassay

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Keywords. Transgenic rat; Hras; mammary tumors; medium-term assay

The abbreviations used are: MNU, *N*-methyl-*N*-nitrosourea; DMBA, dimethylbenzoanthracene; 3-MC, 3-methylcholanthrene; B[a]P, benzo[a]pyrene; IQ, 2-amino-3-methylimidazo[4, 5-*f*]quinoline; MeIQx, 2-amino-3, 8-dimethylimidazo[4, 5-*f*]quinoxaline; NNK, 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone; DEN, diethylnitrosamine; AOM, azoxymethane; DMA, dimethylarsinic acid; PCR, polymerase reaction chain; RFLP, restriction fragment length polymorphisms

Abstract

With the aim of developing a medium-term assay for screening of environmental carcinogens, we exposed mammary carcinogen sensitive human c-Ha-ras proto-oncogene transgenic (Hras128) rats to various carcinogens, including compounds which do not normally induce mammary tumors. Seven-week-old Hras128 rats and wild type littermates received three oral administrations of 3-methylcholanthrene (3-MC), benzo[a]pyrene (B[a]P), anthracene or pyrene (200 mg/kg), 2-amino-3-methylimidazo[*4,5-f*]quinoline (IQ) or 2-amino-3,8-dimethylimidazo[*4,5-f*]quinoxaline (MeIQx)(80 mg/kg), 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or dimethylarsinic acid (DMA)(100 mg/kg), two oral administrations of diethylnitrosamine (DEN)(100 mg/kg), or one oral administration of azoxymethane (AOM)(50 mg/kg) and were killed at week 12 (females) (at week 10 for the 3-MC group) or week 20 (males). Female Hras128 rats receiving NNK, DEN, or DMA showed a significant increase in mammary tumor incidence and/or multiplicity compared to the respective values with olive oil or deionized distilled water (DDW) vehicles. In male Hras128 rats, significant increase in mammary tumors was also observed in groups administered 3-MC, B[a]P, anthracene, IQ, and NNK. Mutations of transgenes were observed in codons 12 and/or 61 in the induced tumors by PCR-RFLP except in the DEN group in female and in the MeIQx group in male Hras128 rats. Thus various carcinogens, not necessarily limited to those normally targeting the breast, were found to induce mammary carcinomas in Hras128 rats, especially in females, pointing to potential use for medium-term screening.

Introduction

We have generated human c-Ha-ras proto-oncogene transgenic (Hras128) rats which are highly sensitive to mammary carcinogens, rapidly developing carcinomas after exposure to *N*-methyl-*N*-nitrosourea (MNU), dimethylbenzo[a]anthracene (DMBA), or PhIP (Asamoto et al., 2000; Tsuda et al., 2001). Furthermore, the Hras128 rats are also highly susceptible to induction of lesions in the esophagus, bladder, skin and tongue (Asamoto et al., 2002; Ota et al., 2000; Park et al., 2004; Suzuki et al., 2005).

Incidence of spontaneous tumors in the mammary gland of Hras128 rats was 52.8% at 40 weeks and slightly increased as compared for female Sprarue-Dawley wild type rats (Tsuda et al., 2005). Taking advantage of these characteristics, we have focused on whether our transgenic animals might have advantages for use in short- or medium-term assay systems for screening environmental carcinogens. One problem is that carcinogens generally have specific organotropic actions as initiating agents (Tsuda et al., 1999). One way to overcome this is to use multi-organ carcinogenesis models (Imaida and Fukushima, 1996; Ito et al., 1988) in which animals are first treated with various carcinogens initiating carcinogenesis in the major organs and then assaying promotion or other modulation effects. However, the established protocols require upwards of 30 weeks until tumors or preneoplastic lesions are induced. As a single organ model, the Ito approach in the liver has many advantages in terms of cost and duration, at 8 weeks, but requires partial hepatectomy to enhance carcinogenesis (Ito et al., 1989; Tsuda et al., 1980). While transgenic (rasH2) mice bearing a human c-H-ras proto-oncogene have attracted interest for testing purposes (Ando et al., 1992; Yamamoto et al., 1996), the assay takes 26 weeks and cannot be said to be short-term. Our Hras128 rats develop tumors within 8 weeks.

For validation in the present study, a number of known carcinogens were selected. These were genotoxic agents as the results could not be applied directly to non-genotoxic agents. The polycyclic aromatic hydrocarbons 3-methylcholanthrene (3-MC) and benzo[a]pyrene (B[a]P) and their parent nuclear substances anthracene and pyrene are included in exhaust gas and tobacco smoke. 3-MC and B[a]P in particular are known to be causative agents for lung cancer in humans and mammary cancers in rats (Bolasny et al., 1963; Gingell et al., 1981). The heterocyclic amines 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3, 8-dimethylimidazo[4, 5-*f*]quinoxaline (MeIQx) are contained in broiled meat and fish (Sugimura, 1985) and 4-(methylnitrosamino)-1-(3-pyridinyl)-1-butanone (NNK) is found in tobacco smoke (Brown et al., 1999). Diethylnitrosamine (DEN) is an N-nitroso compound commonly used in liver cancer experiments (Ito et al., 1989), while azoxymethane (AOM) specifically induces aberrant crypt foci and tumors in the colon (Thorup et al., 1995). Dimethylarsinic acid (DMA) is an arsenic compound present in the environment (Braman and Foreback, 1973) which is known to cause skin, lung and urinary bladder cancers (Chen et al., 1988; Cohen et al., 2001).

In a series of experiments we administered these chemical carcinogens to Hras128 rats and made gross pathological and histopathological assessment of lesion induction. Furthermore, transgene mutations were examined to determine whether the exogenous gene copies were targeted by the carcinogens. The results indicated that the Hras128 rat may indeed have potential for use as a medium-term assay model.

Materials and methods

Animals and chemicals

Sprague-Dawley rats (Clea Japan, Inc., Tokyo, Japan) were used for creating the human c-Ha-ras proto-oncogene transgenic rats (Hras128) (Asamoto et al., 2000), with the human c-Ha-ras proto-oncogene established by Sekiya et al. (Sekiya et al., 1985). The animals were kept under constant conditions with a 12 h light/dark cycle, a room temperature of 22 ± 2°C, and a humidity of 55 ± 10%. They were allowed access to a basal diet (Oriental MF, Oriental Yeast Co., Tokyo, Japan) and tap water. All rats, transgenic and wild type littermates, were treated the same. 3-MC, B[a]P, pyrene, and AOM were purchased from Sigma Chemical Co., St Louis, USA; IQ and MeIQx from Nard Institute, Osaka, Japan; NNK from Toronto Research Chemicals Inc., Ontario, Canada; DEN from Tokyo Kasei, Co., Tokyo, Japan; and DMA and olive oil from Wako Pure Chemical Industries, Osaka, Japan. Anthracene (purity >99.9%) was provided by Dr. Matsushima of Japan Bioassay Research Center, Hadano, Japan.

The experiments were conducted according to the "Guidelines for Animal Experiments in the National Cancer Center Japan" promulgated by the Committee for Ethics of Animal Experimentation.

Experimental protocol (Figure 1)

3-MC, B[a]P, anthracene, pyrene, IQ, MeIQx, and NNK were dissolved in olive oil, and DEN, DMA, and AOM in deionized distilled water (DDW). Two hundred mg/kg of 3-MC, B[a]P, anthracene, and pyrene, 80 mg/kg of IQ and MeIQx, and 100 mg/kg of NNK and DMA were administered by gastric intubation to 7-week-old Hras128 rats and their littermates (wild type) once a week for 3 weeks. One hundred mg/kg of DEN was given once a week for 2 weeks, and 50 mg/kg of AOM once. The control group received 5 ml/kg of olive oil or DDW. Females were killed at week 12, except for the 3-MC treated group (week 10 due to a moribund condition caused by multiple mammary carcinomas), and males at week 20. Numbers, weights and sizes of all mammary tumors were then recorded.

Histological study and DNA isolation

All mammary tumors were removed and, after measurements, were immediately fixed in ice-cold acetone. Tissues were embedded in paraffin and stained with hematoxylin-eosin, followed by histopathological examination. DNA was extracted using DEXPAT (Takara, Otsu, Japan) from paraffin sections 10 m in thickness.

Mutation analysis

Mutation analysis of transgene codons 12 and 61 was performed using the PCR-restriction

fragment length polymorphism (RFLP) approach (Asamoto et al., 2002). The primers for codon 12 were hHras1F (5'-GCAGGCCCCTGAGGAGCGAT-3'), and hHras1RN (5'-AGCAGCTGCTGGCACCTGGA-3'), and for codon 61 were hHras2F (5'-AGCCCTGTCCTCCTGCAGGAT-3'), hHras2R (5'-GGCCAGCCTCACGGGGTTCA-3'), and H61/2A2 (5'-CGCATGGCGCTGTACAGCTC-3'). After 5 min at 95°C, thermocycling conditions were: 1 min at 95°C, 1 min at 60°C, 3 min at 72°C for 35 cycles, with a final extension of 10 min at 72°C. The thermal cycler was a Gene Amp PCR System 9600 (Perkin-Elmer Corp. Norwalk, USA), with MSP I (Takara, Otsu, Japan) for codon 12 and AlwN I (New England BioLabs, MA, USA) for codon 61 as restriction enzymes. After confirming mutations in codons 12 and 61 with PCR-RFLP, DNA lengths of 167 bp for codon 12 and 93 bp for codon 61 were extracted from 4% agarose gels (NuSieve GTG agarose, BMA, USA) using a Min Elute Gel Extraction Kit (QIAGEN, USA) and sequenced using Big Dye Terminator v3.1 (Applied Biosystems, Japan) and an ABI PRIZM3100-Avant Genetic Analyzer (Applied Biosystems, Japan).

Statistics

Analysis of the incidences of mammary tumors and their sizes and multiplicities was conducted using the JMP software package (version 3.1)(SAS Institute, Cary, NC). Chi squared tests were conducted for tumor incidence data and the Dunnett's *t*-test with ANOVA for tumor size and multiplicity.

Results

Incidences and multiplicity of mammary tumors

Female rats (Table 1)

All the tumors taken (larger than 3mm in longer diameter) were adenocarcinomas with obvious invasion of surrouding mammary and stromal tissue. In female Hras128 rats, mammary tumors developed in 7 of 7 rats (100%) given 3-MC, 8/8 (100%) with B[a]P, 4/7 (57.1%) with anthracene, 3/7 (42.9%) with pyrene, 2/10 (20%) with NNK, 7/10 (70%) with IQ, 6/10 (60%) with MeIQx, 3/9 (33.3%) with DEN, 6/9 (66.7%) with AOM, and 1/9 (11.1%) with DMA. There was a significant increase in the tumor incidence in female Hras128 rats in the 3-MC and B[a]P groups at $p<0.001$, the IQ group at $p<0.01$, and the anthracene, MeIQx, and AOM groups at $p<0.05$. The pyrene group also exhibited a significantly increased number of tumors in comparison with the olive oil group ($p<0.05$). Among the littermate rats (wild type), single tumors were found in 2 rats of the 3-MC group and 1 rat of the IQ group, but there were no significant differences from the control (olive oil) group. No tumors other than mammary gland were found in Hras 128 rats. No tumors were detected in any other groups of wild type.

Male rats (Table 2)

The percentages of male rats with mammary tumors for each carcinogen were as follows: 3-MC, 87.5%; B[a]P, 62.5%; anthracene, 42.9%; pyrene, 10%; NNK, 25%; IQ, 16.7%; MeIQx, 8.3%; AOM, 25%; DEN and DMA, 0%. The incidences were significantly increased in the 3-MC, B[a]P ($p<0.001$), and anthracene ($p<0.05$) groups. The multiplicity was significantly increased in the NNK ($p<0.05$) group. Mammary tumor size (7.8±15mm) was significantly greater in the IQ group than in the olive oil group ($p<0.05$). No significant difference from controls was seen in tumor development in the littermate wild rats. In Hras128 rats, zymbal tumors occurred in 3 rats, colonic polyps in 3 rats and scrotal squamous cell papillomas in 2 rats with AOM (Figure 3A), a scrotal squamous cell papillomas in 1 rat and a malignant lymphoma in 1 rat with DMA, zymbal tumors in 2 rats with NNK, scrotal squamous cell papillomas in 2 rats with DEN, a scrotal squamous cell papilloma in 1 rat with IQ and a back skin squamous cell papilloma in 1 rat with pyrene (Table 3). Sarcomas, composed of spindle shaped tumor cells, were found only in male Hras128 rats at lower incidences. These cells were negative for antibodies for pankeratin, S-100 protein and alfa-smooth muscle antigens (Figure 3B). Sarcomas occurred in 2 rats with 3-MC, 1 rat with B[a]p, and 1 rat with MeIQx.

Mutation analysis of the transgenes

The tumors mutation results of PCR-RFLP for codons 12 and/or 61 in the Hras128 rats are shown in Table 3 and 4. Codons 12 and/or 61 in female rats were as follows: 3-MC, 84.8%; B[a]P, 75%; anthracene, 66.7%; NNK, 100%; IQ, 83.3%; and AOM, 100%. Mutations in both codons 12 and 61 were present in 18.2% of the 3-MC group and 28.6% of the B[a]P group (Table 3). Codons12 and/or 61 in male rats were 3-MC, 66.7%; B[a]P, 100%; anthracene, 66.7%; and AOM, 100%. Mutations on both codons 12 and 61 were present in 5.6% of the 3-MC group and 50% of the B[a]P group (Table 4).

Direct sequencing of mutated bands

The results of direct sequencing of DNA are summarized in Table 5. Figures in Table 5 show the numbers of mutation type in mammary tumors in Hras128 rats combined for female with male. In codon 12 there were transversion mutations of GGC to GTC and GGC to TGC (mutation underlined) at rates of 95.3% (61/64) and 4.7% (3/61), respectively. In codon 61 there were transition mutations of CAG to CGG and transversion mutation of CAG to CAT, CAG to AAG, CAG to CTG and CAG to CGT (mutation underlined) at rates of 58.3% (21/36), 33.3% (12/36), 2.8% (1/36), 2.8% (1/36) and 2.8% (1/36), respectively.

Discussion

The present study demonstrated that the mammary tissue of our transgenic rats is sensitive to the carcinogenic actions of chemicals such as IQ, MeIQx, NNK and AOM, the last two not normally inducing breast tumors (Masumura et al., 2003; Reddy et al., 1975; Thorup et al., 1995). Furthermore, positive results were also obtained with 3-MC and B[a]P, along with their parent compounds, pyrene, rated as Group 3 in the IARC Monograph series (1983), and anthracene. It should be noted that anthracene, which has been generally considered as a non-carcinogen, also gave positive results in a 2-year chronic feeding test (personal communication from Dr. Matsushima of the Japan Bioassay Research Center).

Although the mouse model harboring the same human c-Ha-ras proto-oncogene as in our Hras128 rats has been extensively examined for susceptibility to various carcinogens and has found application as a middle-term assay system with lung tumors as the end-point lesions, the experimental protocol required at 26 weeks (Mitsumori et al., 1998; Yamamoto et al., 1998). The duration with the current model, 12 weeks for females and 20 weeks for males has clear advantages in terms of practical application. Indeed, based on our recent observation of development of mammary cancers 15 and 20 days after the administration of MNU (Matsuoka et al., 2003), it may be possible to shorten the experimental period by histopathological detection of early carcinomas in abdominal mammary glands.

Tumors observed in Hras128 were mammary and squamous cell papilloma in the back and the scrotum skin. Histological types of mammary carcinomas were tubular with a cribriform arrangement, solid tubular or papillary tubular (Figure 2), all of which are similarly found after treatment with N-methyl-N-nitrosourea and, importantly, resemble those found in humans (Asamoto et al, 2000). Acinar cell type tumors were not observed. Areas of differing morphology were often found mixed within the same mammary tumors. Furthermore, there was no tendency for specific types to be localized in different mammary glands. No treatment related incidence of any specific histological type or localization was observed. Fibrosarcomas, composed of spindle-shaped irregular shaped tumor cells, were found only in male Hras128 rats at lower incidence. Metastasis from adenocarcinomas was not found. Although we have conducted histological examinations of all major organs, including the esophagus, forestomach, tongue and urinary bladder, which were also found to be highly susceptible to chemical carcinogens in Hras128 rats, no tumors were found, possibly due to the relatively shorter duration of the observation period and low doses of carcinogens. It appears that carcinomas induced in Hras128 rats are not as variable as those observed in transgenic mice (Cardiff et al., 2000).

Since high incidences of the transgene mutation are observed in the mammary tumors in this transgenic rat induced by typical mammary carcinogens (Asamoto et al., 2000), it is clearly of

interest whether the same situation might exist with regard to various other carcinogens. Our present studies clearly indicated that the transgenes, but not the endogenous rat c-Ha-ras gene, demonstrate mutations at relatively high incidence, suggesting an important role in carcinogenesis. Although the number of tumors used for mutation analysis was low except for the B[a]P and 3-MC cases, the results are highly suggestive that the compounds commonly cause mutation of the transgenes. The c-Ha-ras gene was also observed in mice with the same transgene (Ando et al., 1992).

In our recent studies, such mutations were already evident in endbuds (Hamaguchi et al., 2004), postulated tissue targets of carcinogens (Russo et al., 1983; Russo et al., 1979), before obvious proliferative change occurred. Thus it is possible that test compounds including non-mammary carcinogens might also cause mutation of the transgenes, a possibility which we are presently exploring. In the present study, most mutations were of transversion type in codon 12, GGC to GTC predominating, irrespective of the chemical carcinogen. Clearly, it is necessary to analyze whether transversion clustering is dependent on the carcinogen administered or the organ in which the tumor appears.

Establishment of short-term assay models is essential in order to reduce the cost and increase the number of compounds which can be tested (Tennant et al., 1995; Tsuda et al., 1999). From our present review, the human c-Ha-ras proto-oncogene transgenic rat is a good candidate for this purpose. The assay model is advantageous because the end-point is frank mammary carcinomas which can be grossly observed. Furthermore, this model can be used for the assay of modifying agents including chemopreventive compounds (Matsuoka et al., 2003) and also non-genotoxic promoting agents (Fukamachi et al., 2004; Tsuda et al., 2005). Given the number of compounds released in our environment, further validation studies using Hras128 rats will be necessary.

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Figure legends

Figure 1. Experimental protocol for the assay of test compounds using Hras128 and littermate wild type rats.
0 is the start of administration by i.g. and rats are killed at 12 or 20 weeks of this study.

Figure 2.
Macroscopic and histological appearance of tumors found in Hras 128 rats. A, From left, B[a]P-, IQ-, and olive oil-treated female rats. B, Fibroadenoma in a DEN treated female rat. C, Papillary tubular carcinoma in a B[a]P treated female rat. D, Solid tubular (left half) and tubular (right half) carcinoma in an IQ treated female rat. E, Solid tubular carcinoma with a cribriform pattern in an IQ treated female rat. F, Tubular carcinoma with loose fibrosis in a MeIQx treated female rat.
Figure 3
Histological appearance of non-adenocarcinoma tumors. A, Squamous cell papilloma of the scrotum in a Hras128 rat treated with AOM showing papillary formation and keratinization. B, Sarcoma of a mammary gland in a male Hras128 rat treated with B[a]P. Note the spindle-shaped tumor cells showing a storiform arrangement.

Table 1. Incidence and multiplicity of mammary tumors in Hras128 and non-transgenic female rats

	Treatment	No. of rat	Incidence (%)[a]	Diameter(mm)[b]	Microscopic Data		
					Adenoma[b]	Adenocarcinoma[b]	Total[b]
Hras128	3-MC	7	7(100)***	28±8.5**	0.3±0.5	5.3±3.9**	5.6±3.8**
	B[a]P	8	8(100)***	20±8.4**	0.1±0.4	6.8±3.5***	6.9±3.6***
	Anthracene	7	4(57.1)*	9.8±14	0.4±0.5	0.1±0.4	0.6±0.5*
	Pyrene	7	3(42.9)	8.0±8.3	0.1±0.4	0.4±0.8*	0.6±0.8
	NNK	10	2(20)	1.3±9.3	0	0.2±0.6	0.6±0.6
	IQ	10	7(70)**	14±10*	0	2.0±1.9**	2.0±1.9**
	MeIQx	10	6(60)*	7.6±7.1	0	0.8±0.9*	0.8±0.9*
	DEN	9	3(33.3)	4.8±8.3	0.1±0.3	0.2±0.4	0.3±0.5
	DMA	9	1(11.1)	4.5±8.6	0	0.3±0.7	0.2±0.7
	AOM	9	6(66.7)#	13±12#	0	0.6±0.5#	0.6±0.5#
	Olive Oil	11	2(18.2)	6.5±14	0.2±0.4	0	0.2±0.4
	DDW	8	1(12.5)	4.3±8.8	0	0.3±0.7	0.3±0.7
Non-Tg	3-MC	7	2(28.6)	7.2±13	0	0.3±0.5	0.3±0.5
	B[a]P	7	0	0	0	0	0
	Anthracene	8	0	0	0	0	0
	Pyrene	7	0	0	0	0	0
	NNK	12	0	0	0	0	0
	IQ	14	1(7.1)	0.7±2.7	0.1±0.3	0	0.1±0.3
	MeIQx	12	0	0	0	0	0
	DEN	9	0	0	0	0	0
	DMA	9	0	0	0	0	0
	AOM	9	0	0	0	0	0
	Olive Oil	12	0	0	0	0	0
	DDW	8	0	0	0	0	0

a, Adenoma and carcinoma combined; b, Number count / rat, Mean ± SD, *P<0.05, **P<0.01, ***P<0.001 as compared to olive oil of Hras128 rat, #P<0.05 as compared to DDW of Hras128

Table 2. Incidence and multiplicity of mammary tumors in Hras128 and non-transgenic male rats

	Treatment	No. of rat	Incidence (%)[a]	Diameter(mm)[b]	Microscopic Data		
					Adenoma[b]	Adenocarcinoma[b]	Total[b]
Hras128	3-MC	8	7(87.5)***	38±18***	0	4.1±2.9***	4.1±2.9***
	B[a]P	8	5(62.5)***	28±9.8***	0.1±0.4	1.0±0.9**	1.1±1.1**
	Anthracene	7	3(42.9)*	5.5±7.5*	0.3±0.5*	0.1±0.4	0.4±0.5*
	Pyrene	10	1(10.0)	3.0±9.7	0	0.1±0.3	0.1±0.3
	NNK	12	3(25)	3.1±8.1	0.3±0.5*	0	0.3±0.5*
	IQ	12	7(70)**	7.8±15*	0	0.3±0.6	0.3±0.6
	MeIQx	12	6(60)*	4.0±14	0	0.1±0.3	0.1±0.3
	DEN	10	3(33.3)	0	0	0	0
	DMA	10	1(11.1)	0	0	0	0
	AOM	8	6(66.7)#	13±8.5	0	0.3±0.7	0.3±0.7
	Olive Oil	12	2(18.2)	0	0	0	0
	DDW	8	1(12.5)	14.5	0	0.13	0.13
Non-Tg	3-MC	7	1(14.3)	2.7±7.2	0	0.1±0.4	0.1±0.4
	B[a]P	8	0	0	0	0	0
	Anthracene	6	0	0	0	0	0
	Pyrene	10	0	0	0	0	0
	NNK	10	0	0	0	0	0
	IQ	10	0	0	0	0	0
	MeIQx	10	0	0	0	0	0
	DEN	10	0	0	0	0	0
	DMA	10	0	0	0	0	0
	AOM	9	0	0	0	0	0
	Olive Oil	10	0	0	0	0	0
	DDW	8	0	0	0	0	0

a, Adenoma and carcinoma combined; b, Number count / rat, Mean ± SD, *P<0.05, **P<0.01, ***P<0.001 as compared to olive oil of Hras128 rat.

Table 3. Tumors other than mammary glands in Hras128 and non-transgenic rats

	Animals	No. of rat	Number of rats with tumor: Zymbal gland tumor	Colonic adenoma	Scrotum and back skin papilloma	Malignant Lymphoma
Hras128	Female	105	0	0	0	0
	Male	114	5(4.4%)	3(2.6%)	7(6.1%)	1(0.9%)
Non-Tg	Female	117	0	0	0	0
	Male	108	0	0	0	0