Journal of Surgical Oncology 1997;66:97–103

K-*ras* Gene Mutations in Intrahepatic Bile Duct Tumors of Syrian Golden Hamsters

SHIZUO YAMANAKA, MD,[1]* TSUTOMU TOMIOKA, MD,[1] YOSHITSUGU TAJIMA, MD,[1] KAZUYA OKADA, MD,[2] HIROSHI SHIKU, MD,[3] AND TAKASHI KANEMATSU, MD[1]

[1]*Department of Surgery II, Nagasaki University School of Medicine, Nagasaki, Japan*
[2]*Department of Oncology, Nagasaki University School of Medicine, Nagasaki, Japan*
[3]*Department of Internal Medicine II, Mie University School of Medicine, Tsu, Japan*

Background and Objectives: In our laboratory, we have developed a new model of carcinoma of the bile duct in Syrian golden hamsters, using N-nitrosobis(2-oxopropyl)amine (BOP). Morphologic and biologic characteristics of the carcinoma induced in this model are similar to those seen in humans. In order to examine the gene-related carcinogenesis of intrahepatic bile duct carcinoma, we investigated mutations in the K-*ras* gene in various early hyperplastic and neoplastic lesions of these hamsters, according to the original sites of the lesions.

Methods: Inbred female hamsters were given a subcutaneous injection of N-nitrosobis(2-oxopropyl)amine (BOP) following dissection of the extrahepatic bile duct on the distal end of the common duct and preparation of a cholecystoduodenostomy (CDDB) or simple laparotomy (SL). Neoplastic lesions arising from the intrahepatic bile duct were histologically examined, and K-*ras* mutations were investigated.

Results: Mutations of K-*ras* codon 12 were evident in 12% of tubular hyperplasias, 19% of tubular adenocarcinomas, 15% of papillary hyperplasias and 36% of papillary adenocarcinomas. In papillary adenocarcinoma arising from a large bile duct, K-*ras* mutations occurred more frequently than in tubular adenocarcinoma arising from ductule or ductular proliferation. K-*ras* mutations were present even in a hyperplasia; the positive rates of the mutations increased in the presence of a carcinoma. Genetic changes in carcinoma of the intrahepatic bile duct varied based on sites of the duct and the histological type.

Conclusions: A part of the hyperplastic lesions of the intrahepatic bile duct presented K-*ras* gene mutation. This suggests that K-*ras* gene mutation is an early event in the carcinogenic process. In carcinoma of the intrahepatic bile duct, the lesion arising from a large bile duct of the hepatic hilum tended to exhibit a higher frequency of K-*ras* gene mutation than did a tubular lesion arising from ductule or ductal proliferation. This hamster model is useful to examine the carcinogenesis of human intrahepatic bile duct carcinoma.

J. Surg. Oncol. 1997;66:97–103.

KEY WORDS: carcinogenesis; oncogene; chemical carcinogen; cholecystoduodenostomy; hyperplasia

Abbreviations: K-*ras*, Kirsten-*ras;* BOP, N-nitrosobis(2-oxopropyl)amine; BHP, N-nitrosobis(2-hydroxypropyl)amine; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism; CDDB, cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct; SL, simple laparotomy; H&E, hematoxylin and eosin stain.

Contract grant sponsor: Ministry of Education, Science and Culture, Japan; Contract grant number: 06671215.

*Correspondence to: Shizuo Yamanaka, MD, Department of Surgery II, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852, Japan; Fax: (81)-958-49-7319.

Accepted 9 July 1997

INTRODUCTION

In recent years, analyses of malignant tumors at the gene level have been made, and gene-related carcinogenesis has been gradually clarified [1–4]. During the course of development of adenocarcinoma from an adenoma, the activation of a promotor gene and loss of a suppressor gene, such as *p53*, were observed at an early stage of the disease [5,6]. With regard to bile duct carcinoma in humans, Levi et al. [7] reported that Kirsten-*ras* (K-*ras*) mutations were frequent with a sensitive polymerase chain reaction (PCR), while other investigators reported that frequency of such mutations was low [8–11]. Thus, the relationship between K-*ras* mutation and carcinogenesis of the intrahepatic bile duct has remained unclear.

The Syrian golden hamster model has been utilized to investigate the entity of pancreas cancer [12–14]. In our laboratory, a new hamster model of carcinoma in the bile duct was developed using N-nitrosobis(2-oxopropyl)-amine (BOP) [15]. Morphologic and biologic characteristics of the carcinoma induced in this model are similar to those seen in humans [16,17].

To examine this gene-related carcinogenesis of the intrahepatic bile duct carcinoma, we investigated mutation in the K-*ras* gene in various early hyperplastic and neoplastic lesions of these hamsters, according to the original sites of the lesions.

MATERIALS AND METHODS

Animals

Inbred 7-week old female Syrian golden hamsters obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan) were housed in plastic cages on sawdust bedding and given a CE-2 pelleted diet (Japan Clea, Tokyo, Japan) and tap water ad libitum. They were reared under standard laboratory conditions (temperature, 22°C; relative humidity, 40 ± 5%; light/dark cycle, 12/12 hr). All experiments were done in the Laboratory Animal Center for Biochemical Research, Nagasaki University School of Medicine, and this institution's guidelines for Animal Experimentation were closely followed.

Surgery and Administration of Carcinogen

Under sodium pentobarbital anesthesia (50 mg/kg body weight), the hamsters underwent cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct (CDDB) according to our procedures [15]. The control hamsters underwent simple laparotomy (SL). The 61 hamsters that tolerated the procedure were given subcutaneous injections of BOP (10 mg/kg) once a week, starting 4 weeks after surgery, and continued for 9 weeks. Then, 20, 21, and 20 hamsters were killed 12 (CDDB-1), 16 (CDDB-2), and 20 (CDDB-3) weeks after starting the BOP injections, respectively. The 63 control hamsters were given the same BOP treatment; then 20, 22, and 21 hamsters were killed after the same observation periods (SL-1, SL-2, and SL-3).

Tissue Preparation

The liver was immediately removed, fixed in 10% neutral formalin, then cut into five blocks and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) and histologically examined.

Histological Examination

The intrahepatic bile duct carcinomas were classified into four subtypes according to the original sites, as follows [18]: (1) duct infiltrating type, developing from ductar proliferation appearing in medium to large bile ducts; (2) mass forming type, developing from ductule as a scirrhous lesion; (3) intraductal growth type, developing as a papillary elevated lesion of the biliary lumen appearing in the large bile duct near the hepatic hilum; and (4) periductal type, developing from the periductal gland of the biliary wall.

Isolation of DNA

Slides were soaked in xylene and ethanol to remove the paraffin from the surface of the samples. Histologically identified neoplastic lesions were marked; these areas were scraped from the slides and the fragments incubated in buffer (50 mM Tris, 1 mM-EDTA, 0.1% sodium dodecyl sulfate (SDS), 200 μg/ml proteinase K) for 12–24 hr at 37°C. Phenol-chloroform was used to purify the genomic DNA.

Polymerase Chain Reaction–Single-Strand Conformation Polymorphism Analysis

Mutations in exon 1 of the hamster K-*ras* gene were investigated using the following primers specific for the codon 12 and 13 regions of the human K-*ras* gene [3,11]:

Sense primer 5′-GGCCTGCTGAAAATGACTGA-3′
Antisense primer
5′-GTCCTGCACCAGTAATATGC-3′

These primers encompass 162 bp and were synthesized by a DNA synthesizer (model 380B; Applied Biosystems, Foster City, CA). The 5′-end of these primers was labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Thirty-five cycles of polymerase chain reaction (PCR) were programmed as 1 min at 94°C, 55°C, and 72°C, respectively. A portion of PCR products (2 μl) was mixed with 5 ml of 0.1% SDS containing 10 mM EDTA, 7 μl of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue. This mixture was heated at 90°C for 3 min, and applied to a 6% polyacrylamide gel containing 5% glycerol and electrophoresed at 1,800 V for 90–120 min under cooling by a fan in a room at 4°C.

TABLE I. Carcinomas in Intrahepatic Bile Duct of Hamsters After Cholecystoduodenostomy With Dissection of the Distal End of the Common Duct or Simple Laparotomy

Group	No. of hamsters analyzed	No. (%) of hamsters with carcinoma	Average no. of carcinomas per animal	Total no. of carcinomas	Histology			
					Tub	Pap	Peri	Others
CDDB-1	20	15 (75)*	2.00*	40	31	5	1	3
CDDB-2	21	18 (86)**	2.24**	47	33	7	1	6
CDDB-3	20	16 (80)†	4.00‡	80	65	8	1	6
SL-1	20	2 (10)	0.10	2	2	0	0	0
SL-2	22	4 (18)	0.36	8	8	0	0	0
SL-3	21	9 (43)	1.33	28	26	2	0	0

*Significantly different from SL-1 ($P < 0.01$).
**Significantly different from SL-2 ($P < 0.01$).
†Significantly different from SL-3 ($P < 0.05$).
‡Significantly different from SL-3 ($P < 0.01$).
CDDB, cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct; SL, simple laparotomy; Tub, tubular adenocarcinoma; Pap, papillary adenocarcinoma; Peri, adenocarcinoma arising from periductal gland.

TABLE II. Hyperplastic Lesions in Intrahepatic Bile Duct of Hamsters After Cholecystoduodenostomy With Dissection of the Distal End of the Common Duct or Simple Laparotomy

Group	No.of hamsters analyzed	No. (%) of hamsters with hyperplasia	Average no. of hyperplasias per animal	Total no. of hyperplasias	Histology	
					Tub	Pap
CDDB-1	20	19 (95)*	6.05**	121	77	44
CDDB-2	21	21 (100)	6.76	142	93	49
CDDB-3	20	20 (100)	6.90	138	97	41
SL-1	20	10 (50)	2.10	42	28	14
SL-2	22	18 (82)	4.09	90	66	24
SL-3	21	21 (100)	6.00	126	81	45

*Significantly different from SL-1 ($P < 0.05$).
**Significantly different from SL-1 ($P < 0.01$).
CDDB, cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct; SL, simple laparotomy; Tub, tubular hyperplasia; Pap, papillary hyperplasia.

Direct DNA Sequencing

Sequencing primers were end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The DNA fragment obtained with PCR was extracted from the band in the polyacrylamide gel and directly sequenced using the DNA cycle sequencing kit (Takara, Kyoto, Japan). The amplificated reaction mixture was resolved by electrophoresis in 8% acrylamide sequence gel containing 7 mol/L urea.

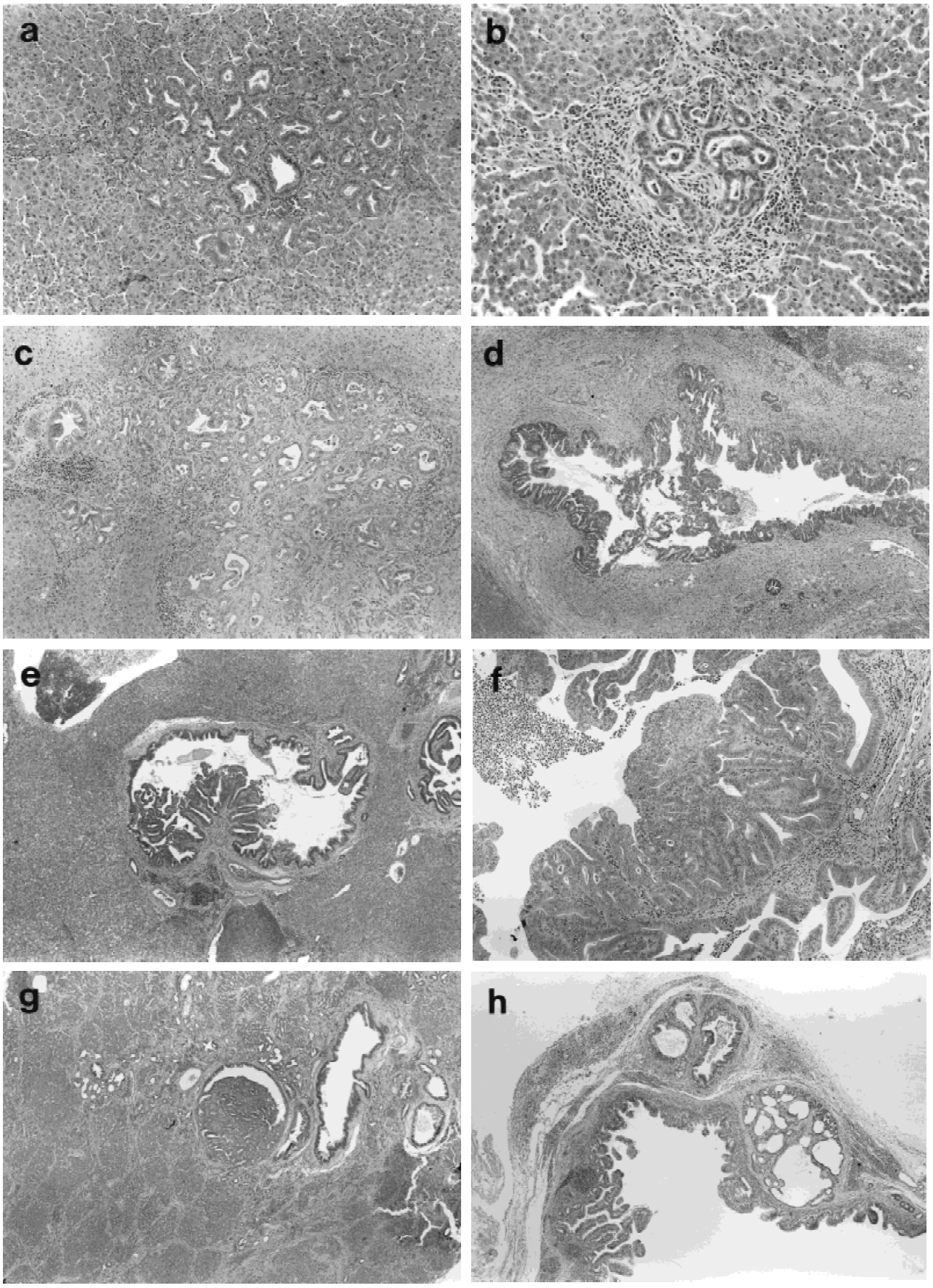
Statistical Analysis

The χ^2 test was used to determine statistical significance in the incidence of carcinoma and hyperplasia production and in the frequency of the K-*ras* mutation. The Mann-Whitney test was also used to compare the average number of carcinomas and hyperplasias per animal. *P* values of <0.05 were considered statistically significant.

RESULTS

Histopathological Findings in the Intrahepatic Bile Duct

Pathological changes advanced with lapse of time and the number of cancerous lesions increased. Intrahepatic bile ducts of hamsters in the CDDB groups showed a high degree of carcinoma development. Cancer was evident in 15 animals (75%) of the 20 animals killed 12 weeks after starting the BOP injection (CDDB-1). When animals were killed 16 (CDDB-2) and 20 (CDDB-3) weeks after starting the BOP injection, cancer was present in 18 of 21 (86%) and 16 of 20 (80%) animals, respectively (Table I). There was only one hamster with neither cancer nor hyperplasia when killed 12 weeks after starting the BOP injection (Tables I, II). The histological type was tubular adenocarcinoma (Table I, Fig. 1c) in 129 of 167 cancer lesions (77%), and 20 lesions (12%) were papillary adenocarcinoma (Table I, Fig. 1f,g). Papillary adenocarcinomas all seemed to be of the intraductal growth type. Among the tubular adenocarcinomas, a scirrhous lesions from the peripheral bile duct (Fig. 1b) was existent, in addition to a lesion advancing from ductal proliferation in the large to medium bile duct (Fig. 1c). However, differentiation was difficult in advanced cases, and only three early scirrhous lesions were identified in all tubular adenocarcinomas. In addition to the above, four cystadenocarcinomas (Fig. 1h) and two mu-


a
b
c
d
e
f
g
h

cinous carcinomas were observed. The incidence of carcinoma and the average number of carcinomas per animal were statistically higher in the CDDB groups than in the corresponding SL groups (Table I). In the CDDB groups, hyperplastic lesions were observed in all hamsters except one killed 12 weeks after starting the BOP injection. The histological type was tubular hyperplasia in 267 of 401 hyperplastic lesions (67%), and 134 lesions (33%) were cases of papillary hyperplasia (Table II, Fig. 1a,d,e). The incidence of hyperplasia and the average number of hyperplasias per animal were statistically higher in the CDDB-1 group than in the SL-1 group, but hyperplastic lesions were present to a high degree also in the SL-2 and SL-3 groups (Table II).

Analysis of K-*ras* Gene Mutations

In the present study, we mainly examined animals which had undergone cholecystoduodenostomy with dissection of the extrahepatic bile duct on the distal end of the common duct, and killed 20 weeks after starting the BOP injection (CDDB-3). We selected for DNA analysis tumor samples which contained very little stromal tissue. K-*ras* exon 1 of neoplastic lesions arising from the intrahepatic bile duct were analyzed by the PCR-SSCP, using the normal bile duct from hamsters not treated with BOP as negative control. An abnormal band or shift was observed in 5 of 26 (19%) tubular adenocarcinomas and 5 of 14 (36%) papillary adenocarcinomas (Fig. 2A). An abnormal band or shift was also observed in 3 of 26 (12%) tubular hyperplasias (Fig. 2B), the early lesion of tubular adenocarcinoma and in 2 of 13 (16%) papillary hyperplasias, the early lesion of papillary adenocarcinoma. Although the number of lesions was small, no mutation was detected in cystadenocarcinoma and tubular adenocarcinoma arising from the periductal gland (Table III). In the case of small scirrhous lesions arising from peripheral ducts, tissues for analysis of K-*ras* gene mutations were too few for a valid analysis.

The direct sequencing method led to detection of a base substitution from GGT(Gly) to TGT(Val) in codon 12 in one tubular adenocarcinoma (Fig. 3, panel 2) and one papillary adenocarcinoma lesion. In other lesions, the base substitution was from GGT(Gly) to GAT(Asp) in codon 12 (Fig. 3, panels 3 and 4). Mutation in codon 13 was not observed in any lesions. Histologically normal portions of the bile duct of hamsters administered BOP were also examined, but no mutation was detected in codon 12 or codon 13. The frequencies of K-*ras* mutation of papillary adenocarcinoma were found to be statistically significant from that of normal duct from BOP-treated hamsters ($P < 0.05$, χ^2 test) (Table III).

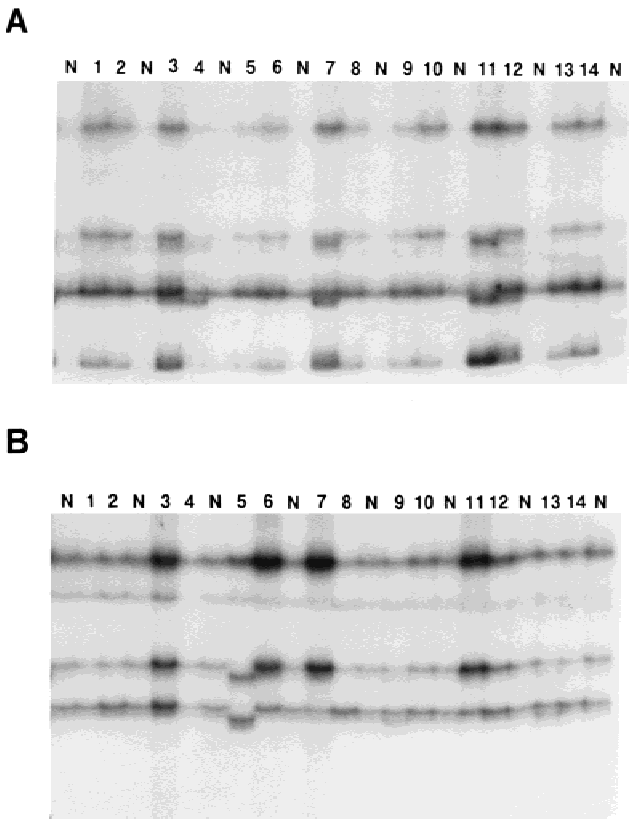


Fig. 2. Single-strand conformation polymorphism analysis of polymerase chain reaction-amplified Kirsten-*ras* exon 1 from intrahepatic bile duct lesions in hamsters. **A:** Papillary adenocarcinoma of intrahepatic bile duct. Lanes 3, 4, 7, 11, 12, shifted bands in addition to normal ones (N). **B:** Tubular hyperplasia of intrahepatic bile duct. Lanes 5, 9, 13, shifted bands in addition to normal ones (N).

DISCUSSION

BOP is a potent carcinogen in the pancreas of the hamster, and the BOP-induced carcinoma resembles that of humans [19,20]. BOP induced intrahepatic bile duct carcinoma has made it possible to observe various early-stage lesions. Such lesions are difficult to observe in humans. In our classification [18], duct infiltrating type and mass forming type are common in human intrahepatic bile duct carcinoma, and we have histologically classified them as tubular adenocarcinomas. In the present study, we mainly analyzed K-*ras* mutations of ductal proliferation and advanced-stage tubular adenocarcinomas. From our laboratory, Yamamoto [21] reported that

Fig. 1. Photomicrographs of intrahepatic bile duct lesions in hamsters. **a:** Ductal proliferation along with accompanying cholangitis around the bile duct. H&E, ×20. **b:** Adenocarcinoma that suddenly developed as a round tumor from ductule. H&E, ×100. **c:** Advanced tubular adenocarcinoma. H&E, ×10. **d:** Papillary hyperplasia with irregular lumen all round. H&E, ×20. **e:** Papillary hyperplasia developing as a papillary elevated lesions from a part of biliary lumen. H&E, ×20. **f:** Papillary adenocarcinoma developing from a large duct near the hepatic hilum. H&E, ×20. **g:** Papillary adenocarcinoma developing from a peripheral duct, a rare occurrence in humans. H&E, ×10. **h:** Cystadenocarcinoma that may develop from a periductal gland in the biliary wall. H&E, ×20.

TABLE III. Analysis of Kirsten-*ras* Codon 12 in Intrahepatic Bile Duct Lesions of Syrian Golden Hamsters

Histology	No. of samples analyzed	No. of mutations detected (%)	Substitutions	
			GGT > GAT	GGT > TGT
Normal duct from BOP-treated hamsters	13	0*	—	—
Tubular hyperplasia	26	3 (12)	3	—
Tubular adenocarcinoma	26	5 (19)	4	1
Papillary hyperplasia	13	2 (15)	2	—
Papillary adenocarcinoma	14	5 (36)**	4	1
Cystadenocarcinoma	2	0	—	—
Tubular adenocarcinoma arising from periductal gland	1	0	—	—

**Significantly different from *($P < 0.05$).
BOP, N-nitrosobis(2-oxopropyl)amine.

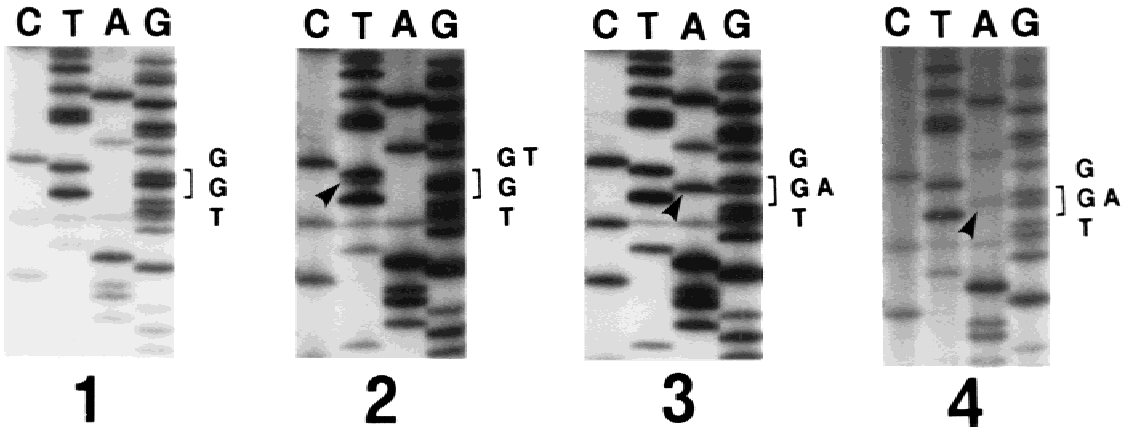


Fig. 3. Direct sequencing of Kirsten-*ras* gene around codon 12 of the intrahepatic bile duct lesions in hamsters. Neoplastic and preneoplastic lesions showing shifted bands in polymerase chain reaction–single-strand conformation polymorphism analysis. **1:** Normal bile duct tissue in hamster. **2:** Tubular adenocarcinoma of intrahepatic bile duct. **3:** Papillary adenocarcinoma of intrahepatic bile duct. **4:** Papillary hyperplasia of intrahepatic bile duct. Arrowheads, point mutated nucleotide. Point mutations from GGT to TGT **(2)** and to GAT **(3 and 4)** were observed.

there was evidence of hyperplasia and hypertrophic changes in periductal glands of patients with intrahepatic stones. Nakanuma et al. [22] reported proliferation and inflammation of periductal glands. Although K-*ras* mutation was not detected in carcinomas arising from the periductal gland in the present study, these reports suggest the possibility of occurrence of intrahepatic bile duct carcinoma in the periductal gland in humans.

The frequency of K-*ras* gene mutation in intrahepatic bile duct carcinoma induced by N-nitrosobis(2-hydroxypropyl)amine (BHP) in hamster was reported to be 25% [23]. The K-*ras* gene mutation in carcinoma of the bile duct in humans was infrequent [7–10]. At first, we expected that the BOP-induced intrahepatic bile duct carcinomas would exhibit a similar incidence and a similar pattern of K-*ras* mutation as the pancreatic cancers [24–26], because BOP might change the same portion of the gene level in the bile and pancreatic duct. However, the incidence of K-*ras* mutations was not so high in intrahepatic bile duct carcinomas as in the pancreatic cancers. The incidence of K-*ras* mutation in intrahepatic bile duct carcinoma or in pancreatic carcinoma in hamsters resembles that in humans.

Our present study revealed that K-*ras* gene mutations were already present in a part of the tubular and papillary hyperplasia lesions, thus K-*ras* gene mutation is an early event in the carcinogenic process. In carcinoma of the intrahepatic bile duct, papillary lesions arising from relatively large bile ducts of the hepatic hilum tended to exhibit a higher frequency of K-*ras* gene mutation than tubular lesions which occurred in ductules, as well as in bile ducts. Motojima et al. [10] in our laboratory reported that K-*ras* gene mutation in carcinoma of the distal portion of the extrahepatic bile duct in humans was more frequent than that occurring in the proximal portion and this is consistent with the results of the present study.

Other workers have reported that a base substitution in codon 12 from GGT(Gly) to GAT(Asp) accounts for most mutations in pancreatic carcinomas and intrahepatic bile duct carcinomas of hamsters and humans [12, 27–29]. In the present study, base substitutions from GGT(Gly) to TGT(Val), a change occasionally observed also in cancers and cell lines of humans [10,27,29,30], was detected in one papillary adenocarcinoma and one tubular adenocarcinoma. In other lesions, including hy-

perplastic ones, base substitution was from GGT(Gly) to GAT(Asp), similar to findings in pancreatic carcinoma in Syrian hamsters [12,23,26]. Regurgitation of pancreatic juice into the biliary tract accompanying anomalous arrangement of the pancreaticobiliary ductal union is considered one factor that contributes to development of biliary carcinoma [31]. In addition to BOP, the regurgitation of pancreatic juice and duodenal fluid is considered to accelerate K-*ras* gene mutation in our hamster model. From histological and genetic aspects, our model is useful to investigate early phenomena of occurrence of bile duct carcinoma in humans.

CONCLUSIONS

A part of the hyperplastic lesions of the intrahepatic bile duct were presented K-*ras* gene mutation. This suggests that K-*ras* gene mutation is an early event in the carcinogenic process. In carcinoma of the intrahepatic bile duct, the lesion arising from a large bile duct of the hepatic hilum tended to exhibit higher frequency of K-*ras* gene mutation than that of the tubular lesion arising from a ductule or ductal proliferation. This hamster model is useful to examine the carcinogenesis of human intrahepatic bile duct carcinoma.

ACKNOWLEDGMENTS

We thank M. Mine, Atomic Bomb Disease Institute, Nagasaki University School of Medicine, Nagasaki, Japan, for help with the statistical analyses, and M. Ohara for reading the manuscript. This work was supported in part by a Grant-in-Aid for General Scientific Research (06671215) from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Kumar R, Sukumar S, Barbacid M: Activation of *ras* oncogenes preceding the onset of neoplasia. Science 1990;248:1101–1104.
2. Sugio K, Kishimoto Y, Virmani AK, et al.: K-*ras* mutations are a relatively late event in the pathogenesis of lung carcinomas. Cancer Res 1994;54:5811–5815.
3. Scarpa A, Zamboni G, Achille A, et al.: *ras*-family gene mutations in neoplasia of the ampulla of vater. Int J Cancer 1994;59:39–42.
4. Tada M, Omata M, Ohto M: Ras gene mutations in intraductal papillary neoplasms of the pancreas. Cancer 1991;67:634–637.
5. Forrester K, Almoguera C, Han K, et al.: Detection of high incidence of K-*ras* oncogenes during human colon tumorigenesis. Nature 1987;327:298–303.
6. Vogelstein B, Fearon ER, Hamilton SR, et al.: Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319: 525–532.
7. Levi S, Urbano-Ispizua A, Gill R, et al.: Multiple K-*ras* codon 12 mutations in cholangiocarcinomas demonstrated with a sensitive polymerase chain reaction technique. Cancer Res 1991;51:3497–3502.
8. Tada M, Omata M, Ohto M: Analysis of *ras* gene mutations in human hepatic malignant tumors by polymerase chain reaction and direct sequencing. Cancer Res 1990;50:1121–1124.
9. Tada M, Yokosuka O, Omata M, et al.: Analysis of *ras* gene mutations in biliary and pancreatic tumors by polymerase chain reaction and direct sequencing. Cancer 1990;66:930–935.
10. Motojima K, Tsunoda T, Kanematsu T, et al.: Distinguishing pancreatic carcinoma from other periampullary carcinomas by analysis of mutations in the Kirsten-*ras* oncogene. Ann Surg 1991;214: 657–662.
11. Tsuda H, Satarug S, Bhudhisawasdi V, et al.: Cholangiocarcinomas in Japanese and Thai patients: Difference in etiology and incidence of point mutation of the c-Ki-*ras* proto-oncogene. Mol Carcinog 1992;6:266–269.
12. Tsutsumi M, Kondoh S, Noguchi O, et al.: K-*ras* gene mutation in early ductal lesions induced in a rapid production model for pancreatic carcinomas in Syrian hamsters. Jpn J Cancer Res 1993;84: 1101–1105.
13. Cerny WL, Mangold KA, Scarpelli DG: K-*ras* mutation is an early event in pancreatic duct carcinogenesis in the Syrian golden hamster. Cancer Res 1992;52:4507–4513.
14. Tomioka T, Fujii H, Egami H, et al.: Correlation between morphology and blood group-related antigen expression in pancreatic tumors induced in Syrian hamsters. Carcinogenesis 1991;12:441–447.
15. Tajima Y, Eto T, Tsunoda T, et al.: Induction of extrahepatic biliary carcinoma by N-nitrosobis(2-oxopropyl)amine in hamsters given cholecystoduodenostomy with dissection of the common duct. Jpn J Cancer Res 1994;85:780–788.
16. Fukahori T, Tomioka T, Inoue K, et al.: Establishment of a transplantable carcinoma arising from the intrahepatic bile duct in Syrian golden hamsters. Virchows Arch A Pathol Anat Histopathol 1993;422:233–238.
17. Inoue K, Tomioka T, Tajima Y, et al.: Characterization of an established transplantable adenocarcinoma of the gallbladder in Syrian golden hamster. J Surg Oncol 1994;56:269–276.
18. Tomioka T, Tajima Y, Ikematsu Y, et al.: The early lesions and invasive patterns of the intrahepatic bile duct carcinoma—comparable study of the hamster and the human lesions. In the Thirty-sixth World Congress of Surgery meeting program, Aug 27–Sept 2, 1995, Lisbon, Portugal, 1995:156, (abst PP39).
19. Pour P, Althoff J, Kruger FW, Mohr U: A potent pancreatic carcinogen in Syrian golden hamsters: N-nitrosobis(2-oxopropyl)-amine. J Natl Cancer Inst 1977;58:1449–1453.
20. Konishi Y, Mizumoto K, Kitazawa S, et al.: Early ductal lesions of pancreatic carcinogenesis in animals and humans. Int J Pancreatol 1990;7:83–89.
21. Yamamoto K: Intrahepatic periductal glands and their significance in primary intrahepatic lithiasis. Jpn J Surg 1982;12:163–170.
22. Nakanuma Y, Yamaguchi K, Ohta G, et al.: Pathologic features of hepatolithiasis in Japan. Hum Pathol 1988;19:1181–1186.
23. Tsutsumi M, Murakami Y, Kondoh S, et al.: Comparison of K-*ras* oncogene activation in pancreatic duct carcinomas and cholangiocarcinomas induced in hamsters by N-nitrosobis(2-hydroxypropyl)amine. Jpn J Cancer Res 1993;84:956–960.
24. Cerny WL, Mangold KA, Scarpelli DG: Activation of K-*ras* in transplantable pancreatic ductal adenocarcinomas of Syrian golden hamsters. Carcinogenesis 1990;11:2075–2079.
25. van Kranen HJ, Vermeulen E, Schoren L, et al.: Activation of c-K-*ras* is frequent in pancreatic carcinomas of Syrian hamsters, but is absent in pancreatic tumors of rats. Carcinogenesis 1991; 12:1477–1482.
26. Ushijima T, Tsutsumi M, Sakai R, et al.: Ki-*ras* activation in pancreatic carcinomas of Syrian hamsters induced by N-nitrosobis(2-hydroxypropyl)amine. Jpn J Cancer Res 1991;82: 965–968.
27. Smit VT, Boot AJ, Smits AM, et al.: KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas. Nucleic Acids Res 1988;16:7773–7782.
28. Grunewald K, Lyons J, Frohlich A, et al.: High frequency of Ki-*ras* codon 12 mutations in pancreatic adenocarcinomas. Int J Cancer 1989;43:1037–1041.
29. Nagata Y, Abe M, Motoshima K, et al.: Frequent glycine-to-aspartic acid mutations at codon 12 of c-Ki-*ras* gene in human pancreatic cancer in Japanese. Jpn J Cancer Res 1990;81:135–140.
30. Berrozpe G, Schaeffer J, Peinado MA, et al.: Comparative analysis of mutations in the *p53* and K-*ras* genes in pancreatic cancer. Int J Cancer 1994;58:185–191.
31. Kinoshita H, Nagata E, Hirohashi K, et al.: Carcinoma of the gallbladder with an anomalous connection between the choledocus and the pancreatic duct; report of 10 cases and review of the literature in Japan. Cancer 1984;54:762–769.