K-ras Mutation is an Early Event in Pancreatic Duct Carcinogenesis in the Syrian Golden Hamster

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ABSTRACT

K-ras oncogene mutation has been shown to be a frequent event in pancreatic ductal adenocarcinomas induced by the carcinogen N-nitroso-bis(2-oxopropyl)amine in the hamster. The present study examines the mutational status of the K-ras oncogene in lesions that precede the appearance of invasive ductal adenocarcinomas. Syrian golden hamsters (80–100 g) received 12 weekly doses of 15 mg/kg N-nitroso-bis(2-oxopropyl)amine and were serially sacrificed at 8, 12, 14, 16, or 24 weeks following the initiation of treatment. Ten μm-thick sections of formalin-fixed paraffin-embedded pancreas were examined for hyperplasia, papillary hyperplasia, carcinoma in situ, and invasive and metastatic ductal carcinoma. Marked lesions of interest were scraped from the slide, subjected to polymerase chain reaction-mediated amplification of the first exon of the K-ras gene, and probed by oligonucleotide-specific hybridization for mutations at codon either 12 or 13. Of 186 samples assayed, K-ras codon 12 mutations were detected in 26% of hyperplasias, 46% of papillary hyperplasias, 76% of carcinoma in situ, 80% of adenocarcinomas, and 43% of lymph node metastases. Codon 12 mutations were exclusively G to A changes at the second position. Codon 13 mutations were only detected in 9 of 168 samples. These results suggest that K-ras activation is an early event in N-nitroso-bis(2-oxopropyl)amine-induced pancreatic carcinogenesis in the hamster.

INTRODUCTION

Despite numerous reports in the literature concerning the detection of activated oncogenes in a wide range of human tumors, little is known about the actual roles these genes play in neoplastic transformation. One approach toward elucidating their role is to determine when during multistage carcinogenesis a particular oncogene is activated. By identifying critical stages during the neoplastic process, it may be possible to unravel the sequence of events required for a particular cell type to become neoplastic. Such analyses ideally involve the identification of the preneoplastic lesions that precede frank tumor formation and have resulted in the identification of several discrete steps in the progression of colon adenomas to adenocarcinomas, involving both oncogene activation (K-ras mutation) and tumor suppressor loss (1, 2). Similarly in mouse skin, analysis of benign papillomas that are believed to precede the development of invasive carcinomas has revealed the activation of 2 different oncogenes (fos and H-ras) (3, 4). Mutation of the K- and H-ras genes prior to the onset of neoplasia has been reported in rat mammary tissue by Kumar et al. (5). These activated oncogenes were detected in morphologically normal mammary cells within 12 days after carcinogen exposure and months before tumor development.

In the present study, we have used this approach to determine whether K-ras activation is an early or late event in the development of BOP3-induced pancreatic ductal adenocarcinoma in the Syrian golden hamster. This animal model has proven to be particularly relevant in the study of pancreatic ductal carcinogenesis due to the fact that the tumors induced are biologically and morphologically strikingly similar to those encountered in humans (6). Recently, it has been shown by our laboratory (7) and others (8, 9) that these tumors frequently contain a mutated K-ras oncogene and, therefore, also share identity with their human counterparts at the molecular level (10–15). Since the majority of patients with pancreatic ductal adenocarcinoma present with advanced disease, early or precursor lesions have very rarely been seen in humans. However, using the Syrian hamster model we were able to observe pancreatic lesions at various stages following BOP treatment and directly assess the status of K-ras activation in focal areas of hyperplasia, papillary hyperplasia, and CIS, as well as in adenocarcinomas and lymph node metastases. Our finding that K-ras activation can be an early event in BOP-induced pancreatic carcinogenesis in the hamster is the first step toward understanding how this oncogene is involved in the neoplastic transformation of pancreatic ducts.

MATERIALS AND METHODS

Carcinogen Administration. Fifty weaning Syrian golden hamsters (80–100 g; Charles River) received s.c. injections weekly with 15 mg/kg of BOP for 12 weeks. Animals were killed at 8, 12, 16, or 24 weeks (experiment I), or 14 weeks (experiment II) following the initiation of treatment, and the pancreas was removed and fixed in 10% buffered formalin.

Tissue Preparation. Two 10-μm-thick sections of formalin-fixed paraffin-embedded pancreas were stained with hematoxylin-eosin and examined for the presence of preneoplastic and neoplastic duct lesions, which included hyperplasia, papillary hyperplasia, CIS, and invasive and metastatic ductal carcinoma. Ductal areas that contained a combination of lesion types were always classified as the most advanced lesion present. (For example, areas where hyperplasia and CIS were contiguous were classified as CIS). Selected lesions were marked and photographed.

Tissue Scraping and Solubilization. Slides were first soaked in xyylene to remove coverslips, then rehydrated in successive washes of absolute ethanol, 95, 80, and 70% ethanol, respectively. Sections were dehydrated in 1% hydrochloric acid in 70% ethanol before a final rinse in water. Marked lesions of interest were scraped from the slide using a 22G needle with special care taken to limit the amount of surrounding normal stroma and acinar tissue. Scraped tissue fragments were placed in 25 μl of buffer (50 mM Tris, pH 8.5, 1 mM EDTA, 0.5% Nonidet P-40 detergent, 200 μg/ml proteinase K). Samples were incubated for 16 to 24 h at 40°C. All samples were heated to 95°C for 10 min prior to being used in the PCR.

Polymerase Chain Reaction. PCR-mediated amplification of a 111-base pair product from the first exon of the hamster K-ras gene in extracted samples was achieved using primers specific for the codon 12 and 13 regions of the human K-ras gene (Clontech Laboratories, Inc., Palo Alto, CA). Forty-five cycles of primer annealing at 37°C (2 min), primer extension at 74°C (2 min), and denaturation at 94°C (1.5 min)
were performed using Taq DNA polymerase (Stratagene, La Jolla, CA) and a Perkin Elmer Cetus Thermal Cycler (Norwalk, CT). Aliquots of PCR were electrophoresed through an 8% polyacrylamide gel, followed by ethidium bromide staining, for analysis.

Oligonucleotide-specific Hybridization. Successful PCR were applied to multiple Zeta-Probe nylon membranes (Bio-Rad Laboratories, Richmond, CA) using a Bio-Dot SF apparatus (Bio-Rad) and a 0.4 M NaOH transfer solution. A set of 7 replicate blots was prepared, one blot for each oligonucleotide probe. A panel of 20mer probes, each specific for a single base change at either the first or second position of human K-ras codon 12 or 13 (Clontech Laboratories), was used for sequence-specific hybridization of the transferred PCR. Oligonucleotide probes were end-labeled with [γ-32P]ATP in a T4 polynucleotidyl kinase labeling reaction. Following hybridization at 37°C, the blots were subjected to stringent washes in a 3 M tetramethylammonium chloride (Aldrich Chemicals, Milwaukee, WI) solution [3 M tetramethylammonium chloride, 50 mM Tris (pH 8.0), 2 mM EDTA, 0.1% sodium dodecyl sulfate] at 68°C. Blots prepared for codon 12 analysis were stripped (0.1 x standard saline-citrate, 0.5% sodium dodecyl sulfate; 95°C for 20 min x 2) and reprobed for codon 13 mutations.

RESULTS

The development of pancreatic ductal adenocarcinoma in the hamster following BOP treatment is marked by a series of well-characterized changes in ductal morphology (16-18) (Fig. 1). The untreated normal ductal epithelium in weanling animals consists of a single layer of flattened cells surrounded by a connective tissue layer (Fig. 1a). The earliest change observed in response to carcinogen exposure is hyperplasia throughout the ductal network (Fig. 1c). Such hyperplasia can become more severe focally and precedes the development of papillary hyperplasia, which is characterized by epithelial outgrowths, some of which are dysplastic, extending into the lumen (Fig. 1d). These changes are observed at the level of the main duct, as well as its smaller tributaries including ductules. Evidence of elongated epithelial fronds with bridging across the lumen in ducts, which maintain an intact basement membrane, is diagnostic of CIS (Fig. 1e). The majority of carcinomas that develop are classified as well-differentiated adenocarcinomas (Fig. 1f).

Examination of pancreata collected at 8-, 12-, 14-, 16-, and 24-week intervals following the initiation of BOP treatment revealed changes in duct morphology that progressed in degree and complexity with time. Organs collected at 8 weeks were marked by mildly hyperplastic ducts with occasional papillary change. Of the 50 lesions marked on sections cut from pancreata taken from 8 of 11 hamsters sacrificed at 12 weeks, approximately half were classified as hyperplasias (26 of 50), with papillary hyperplasias and CIS samples comprising 24% (12 of 50) and 18% (9 of 50) of the samples, respectively. Three primary carcinomas were identified in sections from this time point and metastatic lesions were absent. By 24 weeks, both the number and complexity of the lesions had increased. Examination of tissue sections prepared from 14 animals sacrificed at 24 weeks identified a total of 144 lesions of interest. Notable at this time point was the frequent appearance of lymph node metastases (24 of 144, or 17%) and the increased incidence of carcinomas (24 of 144, or 17%). Preneoplastic lesions (hyperplasia, papillary hyperplasia, and CIS) occurred with approximately equal frequency (31 of 144, 34 of 144, and 31 of 144, respectively).

Lesions from every stage provided suitable template DNA for use in PCRs, resulting in the amplification of a 111-base pair product specific for the codon 12, 13 region of the K-ras gene (Fig. 2). Factors influencing the successful amplification of a particular lesion included the size and cellularity of the tissue sample, as well as the length of formalin fixation prior to paraffin embedding. Although size alone was not always a reliable indicator of the suitability of a sample for PCR, very small lesions (3–4 mm²) of the same stage were generally pooled from a single slide if possible. The frequency of PCR success for each category of lesions was different and, in general, reflected the cellularity of that lesion. For example, hyperplasias demonstrated the lowest frequency of PCR success, with only 46% of the areas scraped resulting in a K-ras specific PCR product. Of all the lesions studied, however, this category had the fewest cells per total area, with the ductal lumen accounting for most of the area within the region marked. On the other hand, papillary hyperplasias, which typically exhibited an increased degree of cellularity as the epithelial outgrowths extended into the ductal lumen, had a higher rate of PCR success, with 66% of these lesions resulting in K-ras specific product. The highest frequency of PCR positive lesions occurred in the CIS and invasive carcinoma categories, where the cells usually filled more of the field of interest. Tissue scraped in these groups resulted in a K-ras specific product following PCR amplification in 74 and 78% of the samples, respectively. PCR success decreased for metastatic lesions (50%), probably due to the cystic nature of some of these samples and the consequential decrease in cellularity. The fact that the frequency of PCR positive samples is less than 100% in all cases can also be attributed to the very small size of some of the areas scraped (<5 mm²) and suggests that there is a lower limit to the sample size for formalin-fixed paraffin-embedded tissue that is suitable for PCR amplification. However, the most important factor affecting the ability of tissue to provide suitable PCR template DNA was the length of formalin fixation. In our experience, tissue that remained in formalin for more than 2 weeks proved to be unsuitable for use in the PCR amplification reaction and consistently failed to generate any product. Tissue fixed for 1 to 7 days worked well in the amplification reaction and as a result, shorter fixation times were chosen for samples collected at 14 weeks in experiment II. Unfortunately, tissue collected in experiment I at the 8- and 16-week time points remained in formalin longer than 2 weeks and therefore was not available for PCR amplification and the subsequent molecular analysis. Overall, 64% of the lesions scraped from the 12-, 14-, and 24-week slides resulted in a K-ras specific PCR product.

Lesions that generated a PCR product were subjected to analysis of K-ras mutational activation at codons 12 or 13 using oligo-specific hybridization. Approximately equivalent amounts of PCR product (as judged by the intensity of ethidium bromide staining) were loaded on slot blots and hybridized with a panel of 7 different oligomers, each specific for a single base change at either the first or second position of codon 12 or codon 13. Since DNA sequencing has revealed that this region of the hamster K-ras gene is very highly conserved and closely matches the human sequence (7, 8), we were able to use human K-ras gene probes to screen our hamster PCR products. A representative set of blots from such an analysis is shown in Fig. 3. The blot hybridized with the probe specific for the G to A change at the first position of codon 12 (serine) is shown in Fig. 3C as an example of the level of background hybridization that was routinely observed. The serine probe usually demonstrated the highest degree of nonspecific binding within the oligomer panel. Fig. 3B shows an example of a blot hybridized...
Fig. 1. Photomicrographs of hematoxylin and eosin-stained sections of hamster pancreatic tissue from untreated control and BOP-treated animals. Lesions are representative of those scraped and probed for K-ras mutations. a, normal pancreatic duct from an untreated animal lined by flat epithelial cells (×188); b, “normal” pancreatic duct from a BOP-treated animal lined by cuboidal epithelium (×188); c, hyperplastic duct in which the epithelium consists of tall basophilic cells (×135); d, papillary hyperplasia with epithelial outgrowths of intensely basophilic and crowded cells (×125); e, CIS (×170); f, invasive well-differentiated adenocarcinoma (×120); g, ductal adenocarcinoma metastatic to a lymph node (×50).

with the aspartate probe for codon 12, and is representative of the range of signal intensities observed.

Oligonucleotide-specific hybridization proved to be a successful and reliable method for evaluating K-ras mutations in the PCR products generated from the focal lesions. Stringent wash conditions eliminated most background binding (Fig. 3C), and positive signals for mutations were identified after assessing the loading concentration of the particular
Fig. 2. PCR-mediated amplification of the first exon of the hamster K-ras gene (111 base pairs) from normal pancreatic duct and various BOP-induced preneoplastic and neoplastic lesions. A 10-μl aliquot from a 100-μl PCR was loaded in each lane. Lanes 1 and 2, untreated pancreatic duct; Lanes 3 and 4, hyperplasia; Lanes 5 and 6, papillary hyperplasia; Lanes 7 and 8, CIS; Lanes 9 and 10, adenocarcinoma; Lanes 11 and 12, metastatic carcinoma; Lane 13, PCR carryover control (no template DNA added); Lane 14, positive control (hamster pancreas DNA); Lane 15, φX174-RF HaeIII digest (500 ng).

Sample as evidenced in the blot probed with the wild-type oligonucleotide (Fig. 3A), and considering the degree of background binding for that particular set of blots. Background binding was estimated by comparing the intensity of the normal pancreas sample (slot 1A) signal on the mutated oligonucleotide blots to its wild type oligonucleotide signal. This ratio was then taken as the threshold of background binding, and all other sample signal ratios had to exceed this number to be considered positive. The only mutations detected in all of the samples analyzed (regardless of stage) were G to A changes at the second position of codon either 12 or 13, with codon 12 mutations occurring much more frequently than those in codon 13. In either case, this mutation results in the substitution of an aspartate residue for a glycine in the ras p21 protein. Positive signals were confirmed by laser densitometry.

Results from the codon 12 oligonucleotide-specific hybridization assay are summarized in Table 1, and are expressed as the percentage of lesions scraped at each stage that were found to contain a mutated K-ras oncogene. Within the group of normal duct lesions, a distinction is made as to the age of the animals from which the samples were taken, although all of the specimens in this category came from hamsters that were not exposed to carcinogen. The first group of 28 samples is from 15 weanling hamsters, while the group designated as “age-matched” represents 16 samples from 10 hamsters approximately 8 months old. These 2 different time points were chosen to assess the background status of K-ras activation in untreated pancreatic ducts in animals of approximately the same ages as those used in the BOP protocol at the beginning (weanling hamsters) and end (8-month-old hamsters) of the study. Regardless of the age of the hamster from which the ductal sample was taken, K-ras mutations were not detected in any of the untreated ducts. The group of 15 samples referred to as BOP “normal” are areas scraped from ducts that exhibited a relatively normal morphology in animals that had received the complete regimen of BOP dosing. It should be noted that very mild hyperplasia is usually observed uniformly throughout the untreated ducts as a result of BOP exposure, and that these samples are representative of such a morphology (Fig. 1b). Of 15 BOP “normal” samples assayed (from 12- and 14-week samples), only one demonstrated a K-ras mutation.

The remaining categories in Table 1 represent the classes of preneoplastic and neoplastic lesions previously described, and include samples from 12-, 14-, and 24-week specimens. Among
these groups, the frequency of \( K-ras \) mutations detected increased as the stage of the lesion became more advanced. Twenty-six \% of hyperplastic lesions were found to contain an activated \( K-ras \) gene, as compared to 46\% of the papillary hyperplasia samples. The percentage of mutated \( K-ras \) positive lesions rose to 76\% for the CIS group, and reached a maximum of 80\% in the primary adenocarcinoma samples. Metastatic lesions demonstrated a \( K-ras \) mutation frequency of 43\%. The frequencies for each group were found to be statistically significant from that of the untreated controls (\( \chi^2 \) test, \( P < 0.01 \)). Within each category of lesions, mutations were found to occur in samples from various time points. Although a majority of the lesions in each group are from 24-week samples (70\% hyperplasia), between 12- and 14-week lesions, we were able to examine the status of \( K-ras \) activation at the codon 12 position as well (1 hyperplasia, 1 papillary hyperplasia, and 2 CIS samples).

**DISCUSSION**

Adenocarcinoma of pancreatic ducts is distinguished by having the highest reported frequency of ras activation of all human tumors studied thus far (19). Over the past 3 years, several investigators have reported mutational activation of the \( K-ras \) gene in 80–90\% of the human pancreatic ductal carcinomas analyzed (10–15). Work in our laboratory (7) and of others (8, 9) has shown that BOP-induced pancreatic ductal carcinomas of the hamster also demonstrate \( K-ras \) gene mutations. The frequent activation of the \( ras \) oncogene in human and carcinogen-induced experimental animal pancreatic ductal adenocarcinomas has stimulated further interest in this oncogene and its role in the process of neoplastic transformation. The use of the experimental hamster model permitted us to examine the ductal lesions that precede the development of gross tumors. By applying PCR amplification to DNA solubilized from these lesions, we were able to examine the status of \( K-ras \) activation throughout the transformation process, beginning with small hyperplastic foci and proceeding through the more advanced stages of papillary hyperplasia and CIS. While the changes that are observed in pancreatic ductal epithelium in response to BOP treatment certainly are distributed along a continuum and include numerous examples of mixed lesions, we imposed these categories as a way to define those morphological changes that appeared to be most consistently associated with progression to carcinoma.

Our ability to successfully amplify the first exon region of the \( K-ras \) gene from focal scrapings was dependent upon several factors. The most critical one that affected all lesions was the length of formalin fixation. Tissue that remained in fixative for 2 weeks or longer proved to be unsuitable for PCR amplification. In our experience, the ideal length of fixation ranged from 24 to 48 h, although tissue fixed for up to 1 week was usable. This effect has been noted by other investigators (20) and is believed to be due to the cumulative effect of nucleoprotein and nucleic acid cross-linking (21).

Analysis of the data summarized in Table 1 suggests that the \( K-ras \) mutation can be an early event during the process of neoplastic transformation in the hamster pancreas. Detection of mutated \( K-ras \) in hyperplasia samples demonstrates that this mutation accompanies the earliest detectable morphological change following carcinogen exposure. The fact that the mutated oncogene is only found in 26\% of the hyperplasia samples can be interpreted in several ways. First, it is important to recognize that although the oligonucleotide hybridization method of identifying mutations is very sensitive, it does have a finite limit of detection. It is possible that we were not able to detect \( K-ras \) mutations where they existed in only a very few cells within a lesion. In preliminary experiments (data not shown), we determined that we could detect a mutated copy of the \( K-ras \) gene when it was present in approximately 5\% of the total DNA blotted. Therefore, we would not detect mutations in any sample where copies of the mutated \( K-ras \) allele fell below this level, and our reported frequencies could represent an underestimate of the true \( K-ras \) mutation frequencies. One might expect that this would be most pronounced in the earliest lesions where, hypothetically, a very small percentage of cells would harbor the mutations presumably caused by carcinogen treatment. Another interpretation of the initial low frequency of \( K-ras \) mutations is that the hyperplastic response itself may represent nonspecific cell replication in response to cell death associated with carcinogen treatment and only the mutation positive lesions will proceed along a neoplastic pathway and eventually result in tumor development. Support for this idea comes from the increasing incidence of \( K-ras \) mutations observed in each subsequent stage of ductal transformation examined in this study, suggesting that lesions that harbor a mutated \( K-ras \) gene are being preferentially expanded. One possible scenario would propose that the \( K-ras \) gene becomes mutated at the second position of codon 12 in response to BOP exposure and that these cells now exhibit some selective advantage that allows for their expansion and enrichment throughout the neoplastic progression towards the development of a pancreatic ductal adenocarcinoma.
The detection of mutated K-ras in a single BOP “normal” sample (1 of 15) raises the possibility that this change may be involved in the first steps of transformation, perhaps initiation, which precedes abnormalities in duct cell morphology; however, a larger number of samples would have to be analyzed to confirm this finding. Unfortunately, these lesions are rare and difficult to isolate in animals that have received the complete schedule of BOP treatment. The absence of mutations in 44 negative control samples (ducts from untreated hamsters in 2 different age groups) argues against the introduction of mutations as a result of Taq enzyme errors generated during PCR amplification.

The observed trend of increasing frequency of K-ras codon 12 mutation accompanying advancing stages of neoplastic progression does not apply to the category of metastatic lesions. For these samples, a decrease in K-ras mutation frequency was found relative to the level seen in invasive carcinomas. This decline may be due to several factors. The cystic nature and florid lymphocyte population of these lesions could contribute to the dilution of cells harboring K-ras mutations to a point below the sensitivity of the oligonucleotide hybridization technique. The lower incidence of K-ras codon 12 mutations may also be due to the random allelic loss of the mutated K-ras in metastatic lesions, and would suggest a passive role for mutated K-ras in metastasis.

K-ras mutations at the codon 13 position were rarely detected (Table 2). Four of the 9 samples that tested positive for codon 13 mutations also contained codon 12 mutations. Because of their very low overall frequency and complete absence in any carcinoma samples, we believe that this mutation is of little consequence in the BOP model of hamster pancreatic carcinogenesis.

Although the detection of an activated K-ras in samples of ductal hyperplasia implicates this mutation as an early event in the process of neoplastic transformation, the assignment of such qualifiers as “early” or “late” are relative to what else is known about the spectrum of molecular changes that are involved in this process. As the first study to address such a question in the hamster model of pancreatic carcinogenesis, this larger frame of reference is certainly lacking. However, we can look to other models in which more extensive research has resulted in the elucidation of molecular changes that accompany neoplastic progression to see that the activation of the ras oncogene as an early event is not unprecedented. H-ras mutations have been detected in a majority of premalignant papillomas arising in the mouse model of dimethylbenzanthracene-induced skin carcinogenesis (3), while K-ras mutations are frequently observed in early to intermediate adenomas that precede colon carcinoma appearance in humans (1). In both of these cases, subsequent molecular events such as additional oncogene activations or suppressor loss have been identified as requirements for the development of the fully transformed phenotype. Both H- and K-ras mutations were found in the mammary tissue of rats exposed to methylmethaneurea within 2 weeks following carcinogen administration, yet these cells appeared to remain latent until exposure to estrogens (5). Therefore, ras activation is postulated to be an early, but not a singularly sufficient, event in transformation.

Recent studies that examine another tumor of the human pancreas, the rare intraductal papillary neoplasm, report conflicting results on the status of K-ras mutation in these lesions. Tada et al. (22) found K-ras mutations in 3 of 5 cases of intraductal papillary neoplasm, and in 100% of adenocarcinomas (19 of 19) (22). However, Lemoine et al. (23) failed to detect any K-ras activations in the 5 cases of intraductal neoplasm that they examined, although they also reported a high frequency of K-ras codon 12 mutation in invasive adenocarcinomas (12 of 16) and intraductal carcinomas with an invasive component (5 of 6) (23). This group also studied 9 cases of ductal papillary hyperplasia, 5 taken from patients diagnosed with chronic pancreatitis and 4 from patients presenting with invasive adenocarcinoma, and failed to detect any K-ras mutations. The authors conclude that the absence of K-ras mutations in the papillary hyperplasia lesions suggests that they may not represent a preneoplastic stage in the development of pancreatic ductal adenocarcinomas in humans, but acknowledge the importance of the mutations in the process of tumorigenesis. Fine needle aspirates of pancreatic masses were screened for K-ras mutations by Shibata et al. (24). Their study detected activated K-ras oncogenes in 72% of patients with malignant cytology (18 of 25), and 25% of patients with atypical cytology (2 of 8), but found no mutations in patients with benign lesions or benign pancreatic disease. Although the detection of mutated K-ras proved to be a good marker for malignant disease, the study is limited to advanced lesions.

The fact that we observe only a single type of K-ras mutation in the ductal lesions, and that this change is found in hyperplasias, implicates this carcinogen as the mutagenic agent responsible for the aspartate mutation. BOP has been shown to be a DNA alkylating agent in the hamster pancreas, primarily producing methyl adducts at the N7 and O6 positions of guanine (25, 26). The guanine in the second position of K-ras codon 12 (GGT) may be particularly sensitive to mutation due to flanking nucleotide residues. Two studies that analyzed mutations induced by N-methyl-N1-nitro-N-nitrosoguanidine showed that guanine residues were preferentially mutated if they had a guanine in the adjacent 5'-position (27, 28). This type of DNA modification is consistent with the observed G to A transition that produces the aspartate mutation in the K-ras gene. The singular nature of the K-ras mutation found in adenocarcinomas and early lesions in the hamster model stands in contrast to the range of mutations reported in human pancreatic adenocarcinoma, where cysteine (8%), arginine (17%), valine (28%), and alanine (3%) substitutions have been reported in addition to aspartate changes (48%) (9–14). These findings reflect the fact that the mutagenic agents that are responsible for the K-ras changes associated with neoplasia and inflammatory disease found in the human pancreas are unidentified and probably numerous. It is striking, however, that although there are a wide range of potential carcinogens that may be responsible for its activation, the K-ras gene is an especially sensitive target in both the human and hamster pancreas. The use of the hamster model of pancreatic carcinogenesis allows us the opportunity to carefully investigate the role of the K-ras oncogene in neoplastic transformation. This report establishes K-ras activation as an early event in this process.

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K-ras in hamster pancreatic carcinogenesis.
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