American Journal of Pathology, Vol. 145, No. 3, September 1994

Pancreatic Carcinomas of Acinar and Mixed Acinar/Ductal Phenotypes in Ela-1-*myc* Transgenic Mice Do Not Contain c-K-*ras* Mutations

Barbara K. Schaeffer, Pamela G. Terhune, and Daniel S. Longnecker

From the Department of Pathology, Dartmouth Hitchcock Medical Center, Lebanon, New Hampshire

c-K-ras is activated by mutation at codon 12 in the majority of human pancreatic carcinomas of ductal but not acinar phenotype. The Ela-1-myc transgene when expressed in transgenic mice induces pancreatic carcinomas of both acinar and mixed acinar/ductal phenotype. The histopathology of 110 pancreatic carcinomas were characterized in this model. A high percentage of the low to moderately differentiated acinar cell carcinomas contain areas of ductal metaplasia. The latter tumors and several well-differentiated acinar tumors were evaluated for c-K-ras mutation to determine whether there is a relationship between the ductal phenotype and c-K-ras mutation. The polymerase chain reaction and allele-specific oligomer hybridization were used to determine whether the c-K-ras gene was mutated at codons 12, 13, or 61. Amplified DNA products from these tumors were also evaluated by single strand conformation polymorphism analysis. Only wild-type c-K-ras was found in these tissues. Not finding c-K-ras mutation in tumors containing ductal morphology indicates that c-K-ras mutation is not a required factor for acinar to ductal metaplasia or a factor in the tumorigenesis of pancreatic tumors that arise in acinar tissue. (Am J Pathol 1994, 145:696–701)

The exocrine portion of the pancreas consists of two major tissue components; 80% acinar and 10% ductal tissue.[1] The majority of carcinomas of the human exocrine pancreas are classified as ductal adenocarcinomas and are considered to be of ductal origin.[2] Of these tumors, a high percentage (75%) contain a mutation in codon 12 of the c-K-*ras* gene.[3] In our laboratory[4] and in that of Lemoine et al[5] the incidence of c-K-*ras* mutation in human carcinomas of the acinar cell type has been found to be much lower. Animal models for pancreatic carcinoma reflect this relationship between c-K-*ras* mutation and cell phenotype. *c-K-ras* activation by mutation at codon 12 is detected in early and late stage ductal adenocarcinomas, arising in the Syrian golden hamster/nitrosamine model.[6–8] However, the c-K-*ras* gene does not contain codon 12 mutation in acinar cell pancreatic carcinomas arising in the rat/azaserine model[9] as well as in the Ela-1-SV40T transgenic mouse model.[10]

Recently, a transgenic mouse model was established in which transformation was directed at the acinar tissue of the pancreas by placing the expression of the c-*myc* gene under the regulation of the murine elastase 1 promoter.[11] Unexpectedly, with increasing age of the transgenic mouse, pancreatic carcinomas of mixed acinar/ductal phenotype and pure acinar phenotype developed. Recent *in vitro* studies of acinar tissue suggest that normal human acinar cells[12,13] are capable of converting to ductal phenotype. The Ela-1-*myc* transgenic mouse line provides an *in vivo* model for determining whether acinar to ductal transdifferentiation is a significant event in pancreatic tumorigenesis. The histopathological characteristics of the pancreas, based on the study of 110 tumors in an Ela-1-*myc* transgenic mouse line, are presented in this report. The status of mutation in codons 12, 13, and 61 of the c-K-*ras* gene is evaluated in DNA extracted from 17 of these tumors, 2 liver metastases, and 1 nontumorous pancreas. We report the absence of mutation in all tumors and metastases including those with ductal cell metaplasia.

Supported by National Institutes of Health Grants CA23108 and CA47327.

Accepted for publication June 12, 1994.

Address reprint requests to Dr. Daniel S. Longnecker, Department of Pathology, Dartmouth Hitchcock Medical Center, 1 Medical Center Drive, Lebanon, NH 03756.

Materials and Methods

Transgenic Mice

Founder pairs from the transgenic mouse line designated as 1195–3, Tg(Ela-1-*myc*)Bri159 were obtained from Dr. Ralph Brinster.[11] Animals were housed and fed as previously described.[14] Male transgenic mice carrying the Ela-1-*myc* transgene were mated with C57Bl/6 × SJL females. The offspring were screened for the transgene using the polymerase chain reaction (PCR). The human growth hormone portion of the transgene was amplified from DNA extracted from tail clips. Primers defined a 95-bp product. The detection of this product in ethidium bromide-stained 2% agarose gels indicated an affected animal.

Tissue Samples and Cell Lines

Tissues for histological study and DNA isolation were harvested from mice of both sexes at the time of autopsy. Individual tumors were dissected free from other tumors and nontumorous pancreas. A portion of each tumor, the rest of the pancreas, and portions of liver and lung were prepared for histological study by fixation in buffered formalin. The remainder of the tumor was frozen at −70 C for DNA isolation. DNA was isolated using a modification of the method described by Sambrook et al.[15] Quantitation was by spectrophotometric analysis. The 110 Ela-1-*myc* tumors were analyzed for histopathological characteristics. Of these, 7 tumors were analyzed for c-K-*ras* mutation. Three of 17 of these acinar cell tumors had no observable ductal component. Two tumors were estimated to be 5 to 10% ductal and the remaining 12 tumors ranged between 15 and 80% ductal.

Positive control DNAs that contain a spectrum of c-K-*ras* mutations that were prepared and referenced previously[10,16] are as follows: Calu-1 with a heterozygous p1 mutation (G → T) in codon 12 and PR310 with a homozygous p3 mutation (A → T) in codon 61, both from human lung carcinomas, and ASPC 1 with a homozygous p2 mutation (G → A) in codon 12 from a human pancreatic tumor and hamster tumor DNA, PDAC, with a heterozygous p2 mutation (G → A) in codon 13. Normal human DNA isolated from circulating blood leukocytes and normal BALB/c mouse DNA isolated from liver served as controls for the wild-type c-K-*ras* gene.

DNA Amplification

All reagents except for bovine serum albumin, magnesium, and the specific primers are contained in the GeneAmp DNA amplification reagent kit (Perkin Elmer Cetus, Norwalk, CT) or from GIBCO BRL (Grand Island, NY). Primers surrounding codons 12 and 13 define a 91-bp fragment of the c-K-*ras* gene. The primers were chosen to be fully homologous to the mouse and the human c-K-*ras* sequence.[17] The sequence of the 5′ primer is 5′GCCTGCTGAAAATGACTGA3′. The sequence of the 3′ primer is 5′TGATTCTGAATTAGCTGTAT3′. Primers surrounding codon 61 define a 94-bp fragment of the c-K-*ras* gene. The sequence of these primers has been published.[9] The human and mouse sequences surrounding codon 61 are identical. Conditions used for PCR, including reagents and thermal profile, have been published.[16]

Allele-Specific Oligonucleotide (ASO) Hybridization

ASO hybridization was performed according to Schaeffer et al.[16] This assay has been shown to be sensitive enough to detect a mutation if it occurs in at least 5% of cells within a population.[18] Probes specific for the wild-type allele, position 1 and 2 base substitutions affecting codons 12 and 13, and position 1 to 3 substitutions affecting codon 61 of c-K-*ras* were included in the analysis. Sequences of the 20-mer oligonucleotide probes for codons 12 and 13 are complementary to the coding strand of exon 1 (codons 9 to 15) of c-K-*ras*.[16] The following are the oligonucleotide probe sequences for exon 2 including codons 58 to 64 for codon 61. Codon 61 is underlined with slashes indicating mixed nucleotides: 61WT probe, 5′TACTCCTCTTGACCTGCTGT3′; 61P1 probe, 5′TACTCCTCTTA/C/TACCTGCTGT3′; 61P2 probe, 5′TACTCCTCTA/C/GGACCTGCTGT3′; 61P3 probe, 5′TACTCCTCC/A/GTGACCTGCTGT3′.

Single Strand Conformation Polymorphism (SSCP) Analysis

SSCP was performed on amplified DNA containing c-K-*ras* codons 12, 13, and 61, as described in Kuhlmann et al.[10]

Sequencing

The modified dideoxy chain termination method of Sanger et al[19] was used for DNA sequence analysis, according to the Sequenase protocol (U.S. Biochemical, Cleveland, OH).

Results

Histopathology

In the pancreas of Ela-1-*myc* mice, carcinomas begin as small groups of phenotypically similar acinar cells (less than 1 mm in diameter) that may show significant failure to achieve normal differentiation. These early foci of atypical cells often develop around islets. Larger neoplasms may show some heterogeneity of cell type with variable degrees of acinar cell differentiation in a single tumor. The acinar cell carcinomas fall into groups as indicated in Table 1. A small fraction are well differentiated (Figure 1). (Note: All photomicrographs are from pancreatic carcinomas of Ela-1-*myc* transgenic mice.) A large fraction shows a low to moderate degree of acinar cell differentiation and contains abundant apoptotic cells (Figure 2). The apoptotic cells were shrunken and separated from surrounding apparently living cells and had condensed nuclear chromatin. These changes were interpreted as death of individual cells in the absence of general changes of necrosis. Another group maintains a moderate degree of acinar cell differentiation but fails to show a significant amount of apoptosis (Figure 3). Rarely these patterns appear in a single tumor so that an isolated area may have conspicuous apoptosis, whereas other areas have little or none. Poorly differentiated acinar cell carcinomas without apoptosis occur but are rare (Figure 4).

A large fraction of tumors develop areas of desmoplasia and ductal metaplasia (Figures 2, 5, and 6). Desmoplasia and ductal metaplasia develop in the low to moderately differentiated acinar cell carcinomas, usually in tumors that show apoptosis. Ductal areas can be the dominant pattern or may represent a small fraction of the total tumor. Thus, the fraction of total tumor that shows a ductal phenotype varies from low (~5%) to high (~95%). No neoplasm of pure ductal phenotype has been identified. A few of the spaces lined by ductal cells become quite large (Figure 7) but none of the neoplasms have been grossly cystic. Ductal metaplasia has not been noted in well-differentiated acinar cell carcinomas.

Table 1. *Phenotypic Classification of Carcinomas of the Pancreas from Ela-1-*myc *Transgenic Mice*

Histological Pattern	N	%
Acinar cell carcinoma		
Well differentiated	8	7.3
Moderate with little or no apoptosis	6	5.4
Poorly differentiated	1	0.9
Poor-moderate with apoptosis	22	20
With ductal metaplasia	73	66.4
Total	110	100

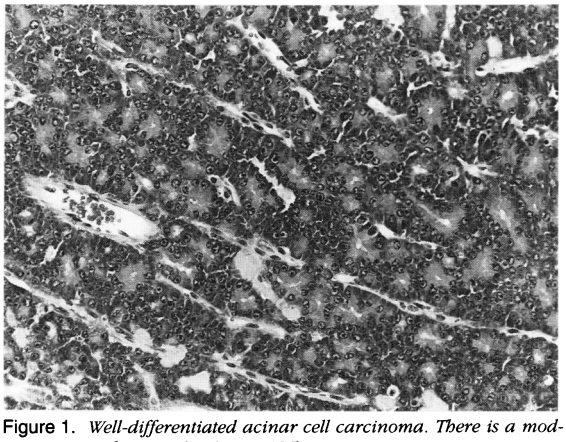

Figure 1. *Well-differentiated acinar cell carcinoma. There is a modest amount of stroma (H & E, ×125).*

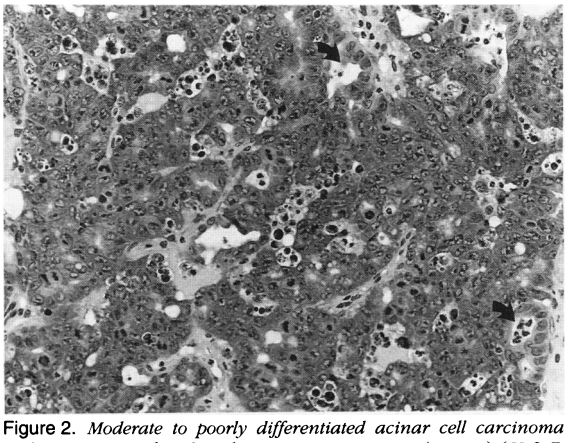

Figure 2. *Moderate to poorly differentiated acinar cell carcinoma with apoptosis. A few ductal structures are present* (arrows) *(H & E, ×125).*

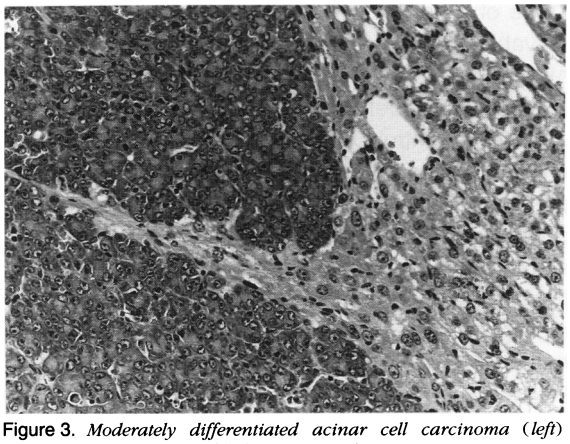

Figure 3. *Moderately differentiated acinar cell carcinoma (left) metastatic to the liver (right) (H & E, ×125).*

Chronic pancreatitis and pancreatic atrophy are frequently noted in the pancreases with large or moderate size carcinomas. It is assumed that this reflects duct obstruction by the tumor, although milder degrees of fibrosis occur in the absence of large carcinomas.

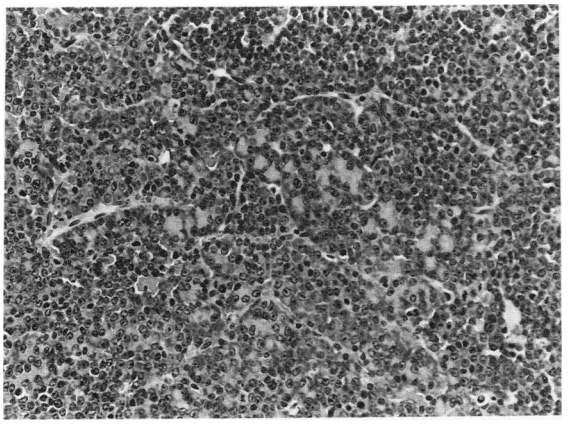

Figure 4. *Poorly differentiated acinar cell carcinoma. The amount of stroma is minimal (H & E, ×125).*

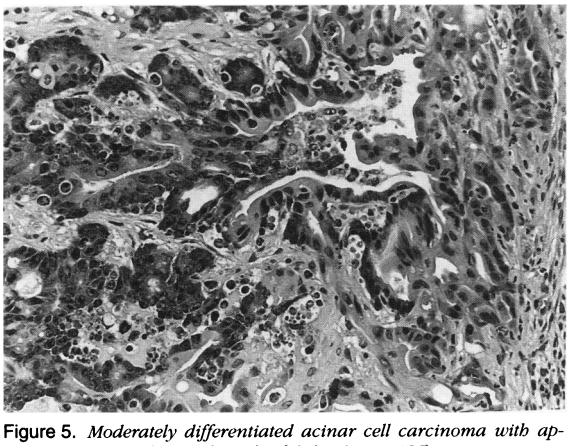

Figure 5. *Moderately differentiated acinar cell carcinoma with apoptosis and ductal metaplasia (right) (H & E, ×125).*

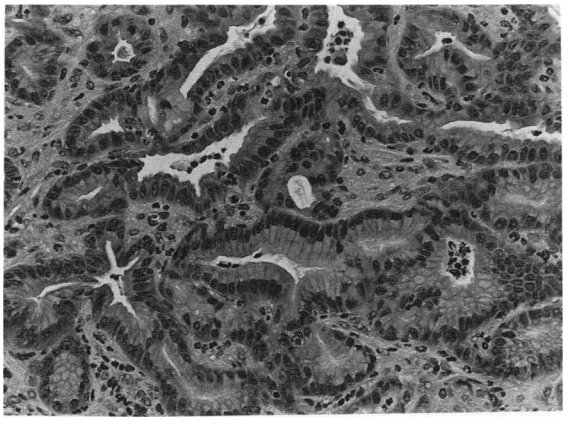

Figure 6. *Area of ductal metaplasia with well-differentiated columnar cells from a carcinoma (H & E, ×125).*

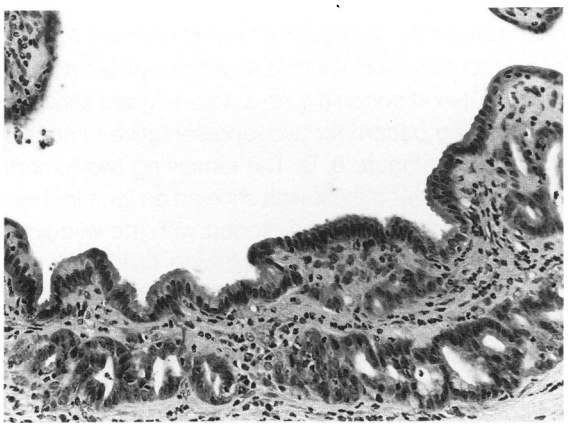

Figure 7. *Area of ductal metaplasia with cyst formation from a carcinoma (H & E, ×125).*

Metastases have been identified in lymph nodes, liver, and lung. Metastatic carcinomas have been moderately differentiated acinar cell type with or without ductal metaplasia (Figure 3).

Status of c-K-ras

Seventeen pancreatic carcinomas, two moderately well-differentiated acinar liver metastases, and one pancreas from an unaffected mouse were evaluated for mutations in codons 12, 13, and 61 of the c-K-*ras* gene. Three of the pancreatic tumors were characterized as well to moderately well-differentiated acinar cell carcinomas and 14 were characterized as acinar cell carcinomas with ductal metaplasia. Ethidium bromide-stained PCR products were observed at 91 bp (K12/13) and 94 bp (K61) positions on 2% agarose gels for all DNAs analyzed. PCR products were transferred to nylon blots. Autoradiograms of these blots probed for wild-type codons 12, 13, and 61 indicated successful transfer of all products as well as the presence of wild-type c-K-*ras* codons 12, 13, and 61 in all DNAs. Autoradiograms of identical blots probed with oligonucleotides containing a mixture of all possible mutations for c-K-*ras* codons 12, 13, and 61 were devoid of signals in those lanes containing transgenic mouse DNA. Control DNAs on each blot yielded signals when hybridized with the appropriate mutated probe, indicating that conditions for hybridization and autoradiography were sufficient for detection of mutation. These negative data are not shown here. Examples of signals obtained for control DNAs *versus* absence of signal for sample DNAs have been published from this laboratory.[10,16]

The above results were confirmed through SSCP analysis. There was no evidence of mutation in codons 12 and 13 in any of the amplified DNAs from transgenic mouse tumors. The banding pattern obtained for amplified DNA from two representative transgenic mouse tumors compared with normal mouse DNA and mutated control DNAs (Calu-1 and ASPC-1) is shown in Figure 8, A. The banding pattern

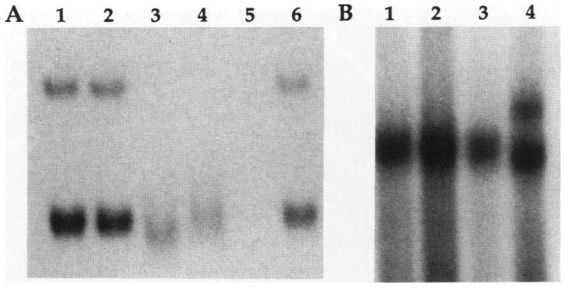

Figure 8. *SSCP analysis was performed on the 91- and 94-bp fragments containing c-K-ras codons 12, 13* (**A**), *and 61* (**B**), *respectively, amplified from the indicated DNA sources. The banding pattern for 2 of the 17 Ela-1-myc tumors along with controls is pictured here.* **A: Lanes 1** *and* **2**, *mixed acinar and ductal cell carcinomas.* **Lane 3**, *ASPC1, human carcinoma.* **Lane 4**, *Calu-1, human carcinoma.* **Lane 5**, *empty.* **Lane 6**, *BALB/c mouse liver.* **B: Lanes 1** *and* **2**, *same mixed acinar and ductal carcinomas as in* (**A**). **Lane 3**, *BALB/c mouse liver.* **Lane 4**, *PR310 human lung carcinoma.*

of amplified DNA for codon 61 from 15 of the 17 transgenic mouse tumors was identical to amplified normal mouse DNA at codon 61. An autoradiogram showing the banding pattern for two representative tumors is presented in Figure 8, B. The remaining two tumors and the two liver metastases showed an identical extra band that did not correspond with the wild-type banding pattern. Direct sequencing of the c-K-*ras* codon 61 PCR products obtained from these tumors confirmed the absence of mutation at codon 61 originally observed by ASO hybridization (data not shown). In addition, the remainder of readable sequence along the PCR fragment did not vary from wild type.

Discussion

The histopathology of pancreatic carcinomas arising in Ela-1-*myc* mice is significantly different than that described in the Ela-1-SV40T transgenic mice.[14] Acinar cell carcinomas of varying differentiation are seen in both models but the spectrum of histological variation is narrower in the Ela-1-*myc* mice. In particular we have not seen examples of the microcystic, macrocystic, and undifferentiated patterns that are seen in exocrine tumors of Ela-1-SV40T transgenic mice. Nor have islet cell tumors been found in the Ela-1-*myc* mice.

We have encountered two patterns that are not seen in Ela-1-SV40T mice. These are the acinar cell carcinomas with ductal metaplasia and acinar cell carcinomas with rampant apoptosis. Ductal metaplasia occurs in acinar cell carcinomas that are moderately to poorly differentiated and show conspicuous apoptosis. We do not see squamous metaplasia in the ductal carcinomas to the degree described by Sandgren et al.[11] The mixed acinar-ductal phenotype has been seen in metastatic foci. This could reflect simultaneous metastatic spread of both cell types or ductal metaplasia in an acinar cell population after metastasis. The latter possibility seems more likely because some of the metastases lack evidence of ductal metaplasia but none shows a purely ductal phenotype.

The data reported here indicate that pancreatic acinar cell carcinomas arising in the Ela-1-*myc* transgenic mice do not contain mutations at codons 12, 13, or 61 of the c-K-*ras* gene. This model, however, differs from the rat/azaserine model[20] and the Ela-1-SV40T transgenic mouse model[21] in that the majority of tumors occurring in mice over 10 weeks of age contain ductal metaplasia.

There are two concerns when analyzing for a mutation in the DNA of a mixed population of cells, in this case acinar, ductal, and stromal cells. Is the percentage of target cell component of the total population of cells high enough for mutation detection? In sampling these tumors for DNA preparation, will the ductal component of the tumor be missed if it represents only a small percentage of the total tissue? In this study, 6 of 12 tumors with ductal metaplasia were more than 25% ductal. If a mutation were present in the ductal cells it would have certainly been detected in these tumors given that the ASO assay is sensitive down to 5%. Using identical conditions for ASO as in this study, the c-K-*ras* mutation was detected within a population of cells, the majority of which were stromal and the minority of which were the target ductal epithelium in small pancreatic adenocarcinomas.[16] The majority of the tumors with ductal components sampled in this study were highly mixed with the central portion of the tumor having a higher fraction of acinar component and the periphery having a predominance of ductal component and fibrous tissue (Figure 5). For these tumors, it seems impossible to have taken a random sample that would consist exclusively of either ductal or acinar components. Thus, the absence of c-K-*ras* mutation in pancreatic tumors that originate in acinar cells but contain neoplastic cells of ductal phenotype suggests that c-K-*ras* mutation is not a factor in the origin or progression of tumors that arise in acinar cells whether or not they undergo ductal cell metaplasia.

References

1. Githens S: The pancreatic duct cell: proliferative capabilities, specific characteristics, metaplasia, isolation and culture. J Pediatr Gastroenterol Nutr 1988, 7:486–506
2. Longnecker DS: Experimental models of exocrine

pancreatic tumors. In The Exocrine Pancreas: Biology, Pathobiology and Diseases. Edited by VLWG Go, FP Brooks, EP Di Magno, JD Gardner, E Lebenthal, GA Scheele. New York, Raven Press, 1986, pp 443–458

3. Shibata D, Capella G, Perucho M: Mutational activation of the c-K-*ras* gene in human pancreatic carcinoma. Bailliere's Clin Gastroenterol 1990, 4:151–169
4. Terhune PG, Heffess C, Longnecker DS: Human pancreatic acinar cell carcinomas contain only wild-type c-K-*ras* codons 12, 13 and 61. Mol Carcinogen. 1994, 10:110–114
5. Hoorens A, Lemoine NR, McLellan E, Morohoshi T, Kamisawa T, Heitz PU, Stamm B, Rüschoff J, Wiedermann B, Klöppel G: Pancreatic acinar cell carcinoma: an analysis of cell lineage markers, p53 expression, and Ki-*ras* mutation. Am J Pathol 1993, 143:685–698
6. Cerny WL, Mangold KA, Scarpelli DG: Activation of K-*ras* in transplantable pancreatic ductal adenocarcinomas of Syrian golden hamsters. Carcinogenesis 1990, 11:2075–2079
7. Fujii H, Egami H, Pour P, Pelling J: Pancreatic ductal adenocarcinomas induced in Syrian hamsters by *N*-nitrosobis(2-oxopropyl)amine contain a c-Ki-*ras* oncogene with a point-mutated codon 12. Mol Carcinogen 1990, 3:296–301
8. Tsutsumi M, Naguchi O, Horiguchi K, Tsujiuchi T, Konishi Y: Ki-*ras* mutation in ductal lesions of hamsters in a rapid production model for pancreatic carcinoma. Int J Pancreas 1992, 12:106
9. Schaeffer BK, Zurlo J, Longnecker DS: Activation of c-K-*ras* not detectable in adenomas or adenocarcinomas arising in rat pancreas. Mol Carcinogen 1990, 3:165–170
10. Kuhlmann ET, Terhune PG, Longnecker DS: Evaluation of c-K-*ras* in pancreatic carcinomas from Ela-I-SV40T transgenic mice. Carcinogenesis 1993, 14: 2649–2651
11. Sandgren EP, Quaife CJ, Paulovich AG, Palmiter RD, Brinster RL: Pancreatic tumor pathogenesis reflects the causative genetic lesion. Proc Natl Acad Sci USA 1991, 88:93–97
12. Hall PA, Lemoine NR: Rapid acinar to ductal transdifferentiation in cultured human exocrine pancreas. J Pathol 1992, 166:97–103
13. Pettengill OS, Faris RA, Bell RH Jr, Kuhlmann ET, Longnecker DS: Derivation of duct-like cell lines from transplantable acinar cell carcinoma of the rat pancreas. Am J Pathol 1993, 143:292–303
14. Glasner S, Memoli V, Longnecker DS: Characterization of the ELSV transgenic mouse model of pancreatic carcinoma: histologic type of large and small tumors. Am J Pathol 1992, 140:1237–1245
15. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual. Edited by N Irwin. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1989
16. Schaeffer BK, Glasner S, Kuhlmann ET, Myles JL, Longnecker DS: Mutated c-K-*ras* in small pancreatic adenocarcinomas. Pancreas 1994, 9:161–165
17. George DL, Scott AF, Trusko S, Glick B, Ford E, Dorney DF: Structure and expression of amplified c-Ki-*ras* gene sequences in Yi mouse adrenal tumor cells. EMBO J 1985, 4:1199–1203
18. Haliassos A, Chomel JC, Grandjouan S, Kruh J, Kaplan JC, Kitzis A: Detection of minority point mutations by modified PCR technique: a new approach for a sensitive diagnosis of tumor-progression markers. Nucleic Acids Res 1989, 17:8093–8099
19. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977, 74:5463–5467
20. Longnecker DS: The azaserine-induced model of pancreatic carcinogenesis in rats. In Experimental Pancreatic Carcinogenesis. Edited by DG Scarpelli, JK Reddy, DS Longnecker. Boca Raton, FL, CRC Press, Inc., 1987, pp 117–130
21. Ornitz DM, Hammer RE, Messing A, Palmiter RD, Brinster RL: Pancreatic neoplasia induced by SV40 T antigen expression in acinar cells of transgenic mice. Science 1987, 238:188–193