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Recent Results in Animal Models of Pancreatic Carcinoma: Histogenesis of Tumors

DANIEL S. LONGNECKER, M.D., VINCENT MEMOLI, M.D., AND OLIVE S. PETTENGILL, Ph.D.

Department of Pathology, Dartmouth Medical School, Lebanon, New Hampshire

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Animal models of carcinoma of the pancreas provide new information regarding the pathways for histogenesis of the tumors. Four models, induced by chemical carcinogens or transgenic methods, are reviewed briefly from this perspective. Recent reports indicate that carcinomas with a ductal phenotype can arise from transformed acinar cells in rodents. A transgenic mouse model provides evidence that anaplastic carcinomas and islet cell tumors may arise from primitive cells that express the elastase gene, yet retain the potential to differentiate as islet cells. In a nitrosamine-induced hamster model, ductal carcinomas appear to arise directly from ductal cells. Carcinomas in this model contained mutations in the c-K-*ras* oncogene that are similar to those reported in about 75 percent of human pancreatic carcinomas, whereas acinar cell carcinomas of rats lacked this mutation. The histologic type of a carcinoma may reflect the cell of origin, but this statement is not always true. Therefore, classification of tumors on the basis of phenotype rather than on the presumed cell of origin is recommended. Among the animal models, the carcinomas in hamster pancreas rank as most similar to human pancreatic ductal adenocarcinomas in regard to the phenotype of the tumors and the prevalence of the c-K-*ras* mutation.

INTRODUCTION

Carcinoma of the pancreas ranks fifth among cancers as a cause of death in the United States [1]. The cancer is usually diagnosed late in its course for several reasons. The internal location of the pancreas makes access for diagnostic studies difficult, and, in most patients, large portions of the gland must be destroyed or obstructed before there are clinical symptoms. Thus, pancreatic carcinomas have often spread to lymph nodes or the liver before the patient seeks medical care, so that surgical removal is difficult or impossible. These characteristics of the disease make its prevention an important goal and, failing prevention, dictate that we need to define the cellular and molecular biology of the carcinomas as a basis for rational approaches to treatment.

Animal models for the induction of pancreatic carcinoma by chemicals or transgenic technology provide models in which preventive approaches can be studied, and in which experimental therapies can be evaluated. These models have provided examples of carcinomas that arise in both acinar cells and ductal cells. The spectra of histologic types in these tumors provide a reasonable match for the tumors encountered in humans.

The evolution of the focal proliferative lesions can be studied in animals by serial

Abbreviations: BOP: N-nitrosobis(2-oxopropyl)amine ELSV: Ela-1-SV40(mice)

Address reprint requests to: Daniel S. Longnecker, M.D. Dept. of Pathology, Borwell 550W, Dartmouth Medical School, Lebanon, NH 03756

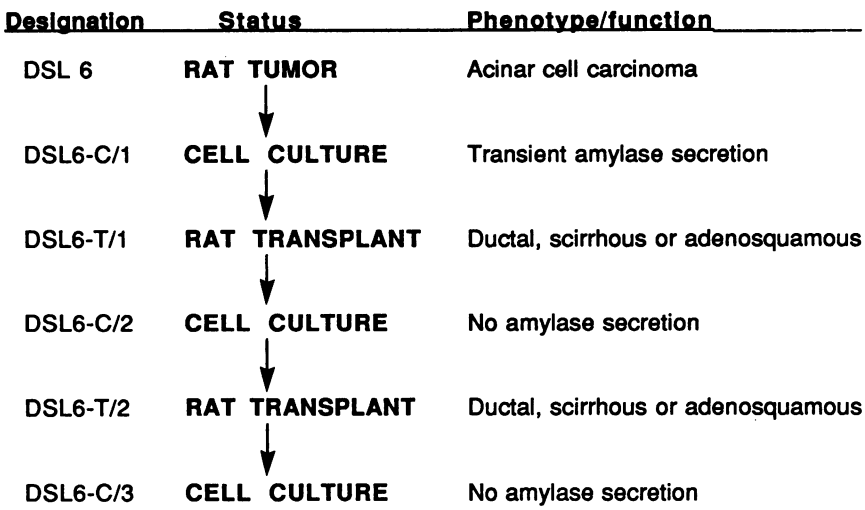

FIG. 1. Flow chart of alternate *in vivo* and *in vitro* growth of cell lines DSL-6A and DSL-6B derived from transplantable acinar cell carcinoma, DSL-6.

autopsies following treatment with a carcinogen, or during the life span of transgenic mice, so that progression from initial cellular changes to neoplasms can be inferred. This approach has provided new perspectives regarding the histogenesis of pancreatic neoplasms in animals, although the relevance of some models for the human has been questioned. In particular, studies reported since 1990 have provided evidence that carcinomas with a ductal phenotype can arise from transformed acinar cells.

ANIMAL MODELS: AZASERINE-INDUCED MODEL IN RATS

Azaserine treatment has provided the best-characterized and most generally used model of pancreatic carcinoma in rats, although similar carcinomas are induced by other agents [2]. Azaserine is an effective pancreatic carcinogen in rats because it is mutagenic and initiates a sequence of focal proliferative changes in acinar cells that culminate in the development of carcinomas [3]. The proliferative sequence begins in acinar cells, and the succession of lesions has been designated as focus, nodule, adenoma, and carcinoma. The development of secondary and even tertiary populations of phenotypically distinct cells within nodules is described [3] and is felt to reflect the stepwise progression to malignancy. The acinar phenotype is retained by the majority of the carcinomas in this model, although focal development of ductlike structures has been noted in a few of the carcinomas. No hyperplastic or dysplastic changes are found in the duct system of azaserine-treated rats, so that it does not appear that neoplasms arise directly from ductal epithelium. The incidence of islet cell tumors in azaserine-treated rats is similar to that in controls.

Recent studies were undertaken to establish and characterize new cell lines from an azaserine-induced acinar cell carcinoma [4]. Two cell lines were established in separate experiments by placing a well-differentiated transplantable carcinoma (DSL-6) derived from a Lewis rat into culture. Although the cultured tumor cells initially produced amylase, production of exocrine enzymes ceased after one to two weeks. The cultured cells were tumorigenic in Lewis rats. The sequence of experiments is outlined in Fig. 1. One cell line produced firm, solid tumors with a high

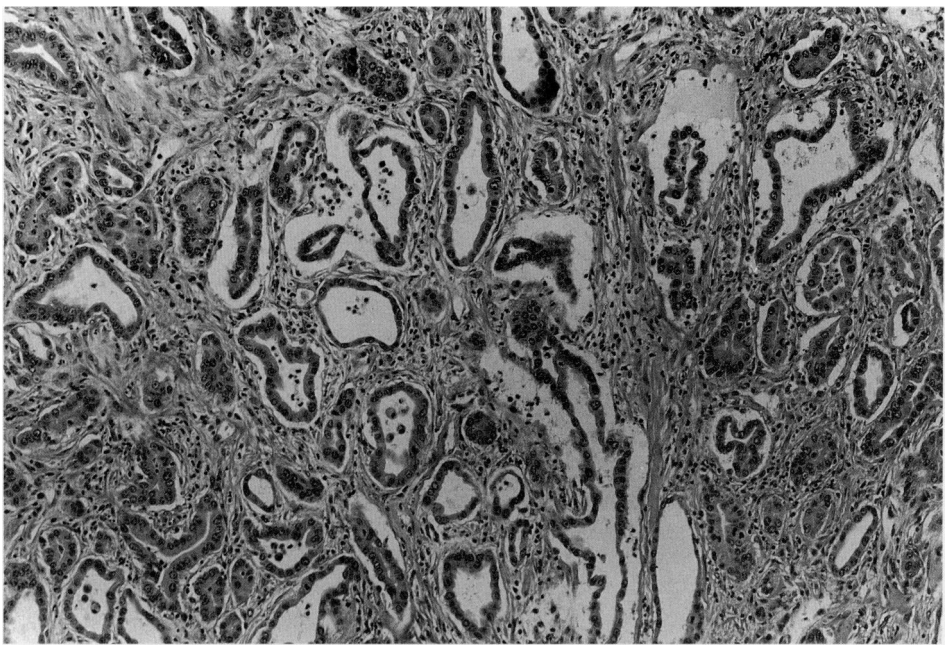

FIG. 2. Subcutaneous tumor produced by inoculating the first cell line, DSL-6A, into a syngeneic rat. The tumor is composed of ductlike structures separated by dense fibrous tissue. Hematoxylin and eosin. ×120.

content of fibrous tissue surrounding ductlike structures when it was grafted into syngeneic rats (Fig. 2), and the second cell line yielded tumors that grew with an adenosquamous phenotype—producing a significant amount of mucin. In each case, the altered phenotype persisted when the tumors were returned to culture (Fig. 3), and then re-implanted into rats.

The original tumor had a high content of CCK receptors (radioligand binding assay) [5], but the first cell line established in culture lacked the receptors (the second cell line has not been evaluated for the presence of receptors). Electron microscopy showed ductlike cells without zymogen granules and with little rough endoplasmic reticulum. Immunohistochemical studies of the cell lines and the re-grafted tumors have demonstrated expression of several ductal markers, including cytokeratin 19. These studies provide strong support for the hypothesis that ductlike carcinomas can arise from neoplastic pancreatic acinar cells in rats. There is loss of acinar cell differentiation and acquisition of ductal markers in the tumor cells.

TRANSGENIC MOUSE MODELS

Transgenic mice that express several growth-controling genes in the pancreas provide new models for pancreatic carcinogenesis. Two strains of transgenic mice bearing the elastase promoter-SV40-early antigen construct (Ela-1-SV40 T), designated as Tg(Ela-1, SV40E)Bri18 and Tg(Ela-1, SV40E + Ela-1, neo)Bri19 [6], and another strain bearing the Ela-1-myc construct, designated as Tg(Ela-1, Myc)Bri159 [7], are the best characterized. The transgenes for these strains utilize the elastase-1 enhancer/promoter to target oncogene expression to the exocrine pancreas. These mice have been used for *in vivo* characterization of focal neoplastic transformation

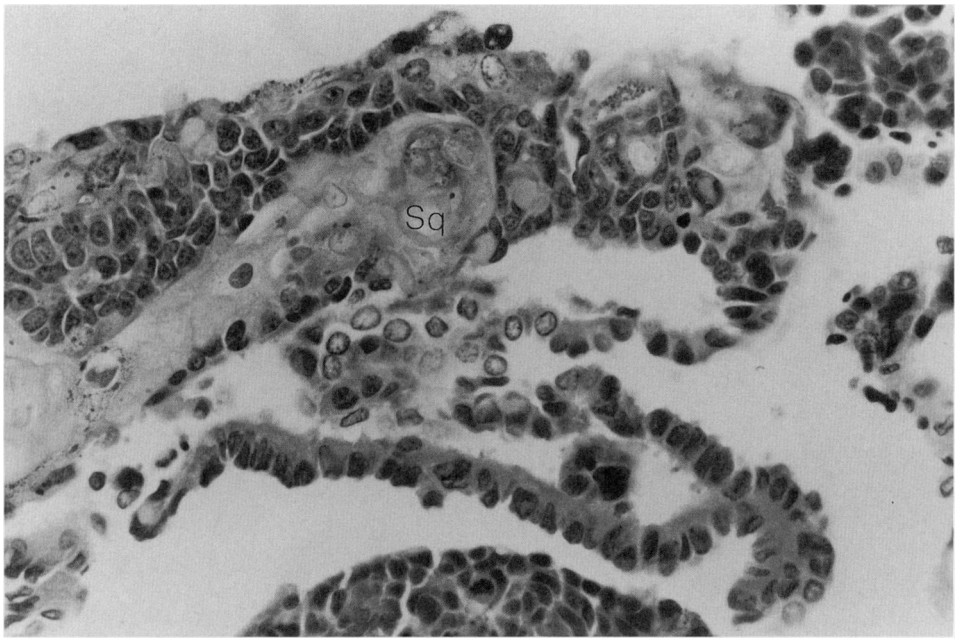

FIG. 3. Cultured cells of the second line, DSL-6B, showing both glandular and squamous (**Sq**) differentiation. This cell line formed adenosquamous carcinoma when it was re-grafted into syngeneic rats. Hematoxylin and eosin. ×630.

and tumor progression in the pancreas [8,9]. Two features of these models have special relevance for the histogenesis of pancreatic neoplasms.

The Ela-1-SV40 T (ELSV) mice develop diffuse acinar cell dysplasia and hyperplasia, followed by focal acinar cell proliferative lesions that develop into carcinomas. Most carcinomas retain evidence of acinar cell differentiation, as in the azaserine model in rats, but diverse histologic types of cancer including undifferentiated (anaplastic) tumors occurred [9]. An unpredicted high incidence of islet cell tumors developed in the Bri18 strain mice [10]. A novel form of islet hyperplasia was also found in the majority of these mice and was implicated in the pathogenesis of the islet cell tumors. The abnormal islets were composed of a core of normal, mature-appearing islet cells that contain insulin identified in immunohistochemical stains. The core is surrounded by a mantle of small cells that stained intensely for somatostatin by immunohistochemistry. Expression of T antigen was not originally detected in β-cell tumors [10], but recent immunohistochemical studies (unpublished) in our laboratory indicate that T antigen is expressed in the nuclei of some of the small peripheral cells in hyperplastic islets and in some small islet cell tumors (Figs. 4 and 5).

These findings suggest that some of the tumors that were originally classified as undifferentiated (small-cell) carcinomas may arise from the same cells as the small cells at the periphery of the abnormal islets in the Bri18 mice, and that the resulting tumors may either remain as anaplastic tumors composed of small cells or differentiate into islet cell tumors. One interpretation is that a stem cell population expresses the Ela-1-SV40 T transgene and therefore proliferates. This population could give

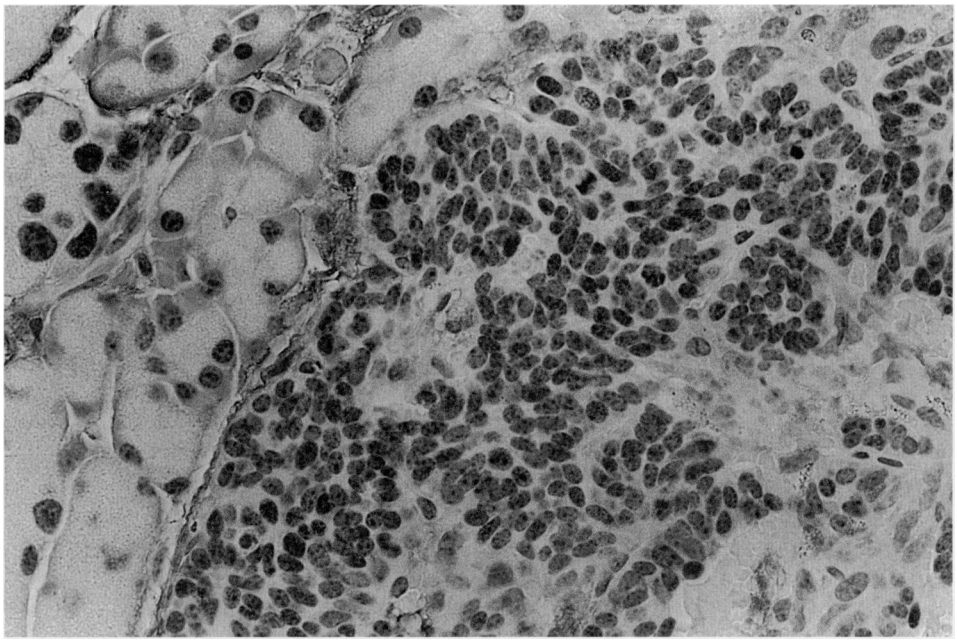

FIG. 4. Peripheral portion of a large hyperplastic islet from a Tg(Ela-1, SV40E)Bri18 transgenic mouse. Dysplastic acinar tissue occupies the left third of the field. Acinar and islet cells with the darkest nuclei express T antigen in the nucleus. Immunoperoxidase stain, monoclonal anti-T-antigen antibody. ×480.

rise to the mantle of somatostatin-positive cells that is seen around some islets in this model, to anaplastic carcinomas, or to islet cell tumors.

Ela-1-myc transgenic mice show many similarities to Ela-1-SV40 T mice and develop diffuse hyperplasia of acinar tissue, followed by acinar cell carcinomas of the pancreas. This strain displays two features that were not observed in Ela-1-SV40 T mice. About half of the acinar cell tumors contain areas of ductal differentiation associated with desmoplasia [7]. The ductal elements in some of these carcinomas have undergone squamous metaplasia, producing an adenosquamous pattern. The proliferative potential of the ductal areas appears to be limited, so that the ductal phenotype does not become dominant, and no purely ductal carcinomas have been described. The observations in this model may supply an additional example of metaplasia of transformed acinar cells to a ductal phenotype, although it is possible that the ductal component is derived from an unrecognized population of epithelial stem cells.

NITROSAMINE-INDUCED MODEL IN THE HAMSTER

N-nitrosobis(2-oxopropyl)amine (BOP) is a potent pancreatic carcinogen in hamsters. BOP is metabolized to an alkylating species that is mutagenic and capable of initiating a carcinogenic sequence, yielding carcinomas that usually have a ductal phenotype. Atypical papillary epithelial hyperplasia has been described focally in the pancreatic ducts of BOP-treated hamsters, and this type of change is regarded as the origin of at least some of the carcinomas. Focal proliferative lesions in the lobules of

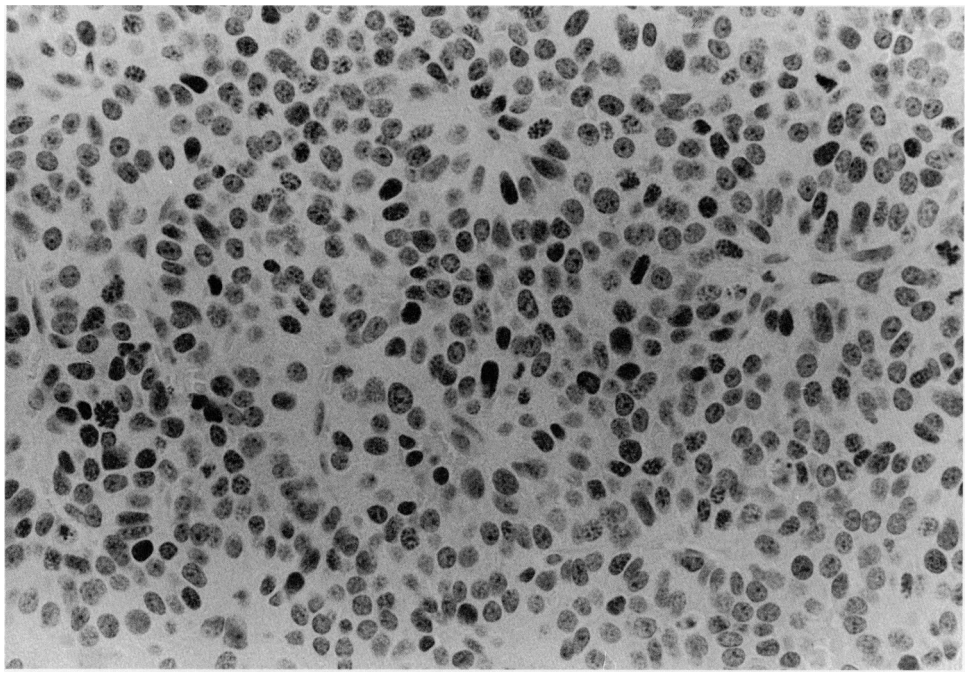

FIG. 5. Islet cell tumor from the pancreas of a Tg(Ela-1, SV40E)Bri18 transgenic mouse. The darker nuclei are stained positively for T antigen. Immunoperoxidase stain, monoclonal anti-T-antigen antibody. ×480.

the pancreas, termed *tubular ductal complexes* or *pseudoductular hyperplasia,* are also regarded as possible early lesions in a carcinogenesis sequence. The BOP-induced model in hamsters has been widely studied and utilized because of the similarities of the ductal carcinomas to the most common type of pancreatic carcinoma found in humans [11].

ONCOGENE CHANGES IN EXPERIMENTAL CANCERS

Recent studies indicate that about 75 percent of human pancreatic carcinomas have an activated c-K-*ras* oncogene, usually with a mutation in codon 12 [12]. A similar codon 12 mutation has been found in the majority of hamster carcinomas that have been evaluated, but a smaller number of hamster tumors have had a codon 13 mutation [13,14,15]. Recent studies indicate that the c-K-*ras* mutation occurs early during carcinogenesis in the hamster model [16].

The azaserine-induced acinar carcinomas of rats have been found to contain wild-type c-K-*ras* when examined for mutations at codons 12, 13, and 61, and they thus appear to lack the *ras* mutations that most commonly activate the proto-oncogene in humans [15,17]. Acinar cell tumors induced in rats by pancreaticobiliary diversion similarly lack the c-K-*ras* mutations [18]. van Kranen et al. also evaluated azaserine-induced acinar cell carcinomas for H-*ras* mutations, and none were found [15]. Preliminary studies indicate that the ELSV mouse carcinomas also lack mutation in codon 12 of c-K-*ras* [Longnecker DS, Kuhlmann ET: unpublished].

DISCUSSION

Avenues for the histogenesis of pancreatic carcinoma have been outlined on the basis of data from several animal models. These pathways indicate that acinar cells may be transformed in rats and mice and may give rise to acinar cell carcinomas, ductlike carcinomas, adenosquamous carcinomas, and undifferentiated carcinomas. Alternately, duct or centroacinar cells may be transformed in the hamster model to give rise to ductlike carcinomas and other histologic variants. These studies have demonstrated that the spectra of histologic types of pancreatic cancer arising from acinar and ductal cells overlap. Thus, the morphologic classification of a pancreatic carcinoma does not necessarily reflect the histogenesis, as has been noted for neoplasms arising in other organs [19]. Classification on the basis of tumor phenotype, e.g., ductal, acinar, islet, anaplastic, is more accurate. Moreover, the expression of more than one differentiated phenotype in a tumor may be evidence for the transformation of a primitive (stem) cell.

In hamster, rat, and mouse models, development of the carcinomas is promoted by feeding the animals a high-fat diet, but the mechanism of this effect is not known [20]. In rats and mice but not in hamsters, exocrine carcinomas have a higher incidence in male than in female animals, as is true in the human. Testosterone seems to support, and estrogen to suppress, the development of the carcinomas in these two rodent species. Several peptide hormones have also modified carcinogenesis in the rat [21]. Thus, carcinogenesis in the pancreas appears to be a complex process that can be influenced by exposure to chemical carcinogens, dietary factors, and endogenous hormonal factors.

Mechanisms of the effect of dietary factors and hormones on pancreatic carcinogenesis can be studied in the animal models, and they can be used to study the effects of exogenous hormonal treatment on established cancers. In such studies, relevance to the human is always a question, and comparative studies with human carcinomas and pancreatic cancer cell lines are important. Among the animal models, the pancreatic carcinomas in hamsters rank as most similar to human pancreatic ductal adenocarcinomas in regard to the phenotype of the tumors and the prevalence of the c-K-*ras* mutation. On the other hand, some of the less common histologic types of human pancreatic cancer, e.g., acinar cell carcinoma, are similar to the carcinomas found in rat and mouse models. When all factors are considered, there appears to be a continuing role for study of pancreatic cancer in several rodent species with ongoing comparison to human tumors. A major focus for the immediate future is to extend the comparison at the molecular level and to define genetically determined abnormalities of growth control.

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REFERENCES

1. Boring CC, Squires TS, Tong T: Cancer statistics. Cancer 42:19–38, 1992
2. Longnecker DS: Experimental models of exocrine pancreatic tumors. In The Exocrine Pancreas: Biology, Pathobiology and Diseases. Edited by VLW Go. New York, Raven Press, 1986, pp 443–458
3. 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
4. Longnecker DS, Faris RA, Bell RH Jr, Kuhlmann ET, Pettengill OS: Ductal metaplasia in cell lines derived from an acinar cell carcinoma of the rat pancreas. Pancreas 6:710, 1991
5. Bell RH Jr, Kuhlmann ET, Jensen RT, Longnecker DS: Overexpression of cholecystokinin receptors in azaserine-induced neoplasms of the rat pancreas. Cancer Res 52:3295–3299, 1992
6. 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 238:188–193, 1987
7. Sandgren EP, Quaife CJ, Paulovich AG, Palmiter RD, Brinster RL: Pancreatic tumor pathogenesis reflects the causative genetic lesion. Proc Natl Acad Sci USA 88:93–97, 1991
8. Longnecker DS, Kuhlmann ET, Freeman DH Jr: Characterization of the elastase-1 SV40 T-antigen mouse model of pancreatic carcinoma: Effects of sex and diet. Cancer Res 50:7552–7554, 1990
9. Glasner S, Memoli V, Longnecker DS: Characterization of the ELSV transgenic mouse model pancreatic carcinoma: Histologic type of large and small tumors. Am J Pathol 140:1237–1245, 1992
10. Bell RH Jr, Memoli VA, Longnecker DS: Hyperplasia and tumors of the islets of Langerhans in mice bearing an elastase I-SV40 T-antigen fusion gene. Carcinogenesis 11:1393–1398, 1990
11. Pour PM, Egami H, Takiyama Y: Patterns of growth and metastases of induced pancreatic cancer in relation to the prognosis and its clinical implications. Gastroenterology 100:529–536, 1991
12. Shibata D, Capella G, Perucho M: Mutational activation of the c-K-ras gene in human pancreatic carcinoma. Baillieres Clin Gastroenterol 4:151–169, 1990
13. Cerny WL, Mangold KA, Scarpelli DG: Activation of K-ras in transplantable pancreatic ductal adenocarcinomas of Syrian golden hamsters. Carcinogenesis 11:2075–2079, 1990
14. 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 3:296–301, 1990
15. van Kranen HJ, Vermeulen E, Schoren L, Bax J, Woutersen RA, van Iersel P, van Kreijl CF, Scherer E: Activation of c-K-ras is frequent in pancreatic carcinomas of Syrian hamsters, but is absent in pancreatic tumors of rats. Carcinogenesis 12:1477–1482, 1991
16. Cerny WL, Mangold KA, Scarpelli DG: Activation of K-ras during N-nitrosobis(2-oxopropyl)amine induced pancreatic carcinogenesis. Proc Am Assoc Cancer Res 32:138, 1991
17. Schaeffer BK, Zurlo J, Longnecker DS: Activation of c-K-ras not detectable in adenomas or adenocarcinomas arising in rat pancreas. Mol Carcinogen 3:165–170, 1990
18. Hall PA, Lemoine NR, Murphy G, Dowling RH: Molecular differences between human and experimental pancreaticobiliary diversion-induced rat pancreatic neoplasia. Gut 32:533–535, 1991
19. Gould VE, Memoli VA, Dardi LE: Multidirectional differentiation in human epithelial cancers. J Submicro Cytol 13:97–115, 1981
20. Longnecker DS: Experimental pancreatic cancer: Role of species, sex and diet. Bull Cancer 77:27–37, 1990
21. Longnecker DS: Hormones and pancreatic cancer. Int J Pancreatol 9:81–86, 1991