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Animal models of exocrine pancreatic carcinogenesis

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Abstract

In order to understand the evolution, histogenesis, and biological behaviour of exocrine pancreatic carcinoma, some reproducible experimental models have been developed in certain rodent species. To date, more than 16 chemicals, many of them structurally unrelated, have been shown to induce pancreatic tumors. Although some of these chemicals appear species specific in their effect on the pancreas, others have been shown to be capable of inducing pancreatic tumors in more than one species. In hamsters, the administration of diisopropylnitrosamine or its oxidized metabolites leads to the development of ductal adenocarcinomas that histologically resemble human pancreatic carcinomas. The histogenesis of the ductal type of adenocarcinoma in hamsters is complex, and appears to involve both the duct cells and dedifferentiated acinar cells. All pancreatic tumors in rats develop from acinar cells showing variable degrees of differentiation, regardless of the type of carcinogen used. The type of pancreatic lesions that develop in mice are also of acinar cell origin. In guinea pigs the tumors are adenocarcinomas of the ductal type and are shown to be derived from dedifferentiated acinar cells that have undergone duct-like transformation. Irrespective of the type of tumor that develops in these experimental animals, all of these models can be successfully used to evaluate the various modifying (risk) factors and biological behaviour of these neoplasms.

Introduction

In the United States, carcinoma of the exocrine pancreas ranks fifth among all cancers and is the fourth leading cause of cancer deaths [1]. Human pancreatic carcinoma, which causes approximately 25,000 deaths a year, is considered a dismal disease because of poor prognosis [1, 2]. The increased mortality of this disease is due to late diagnosis, local and distant metastasis at the time of initial clinical manifestation, and poor understanding of the biological behaviour of this tumor. Although the etiology of pancreatic carcinoma is not known, epidemiological studies have indicated several risk factors such as alcohol, cigarette smoking, and certain nutritional factors [3–5]. However, these epidemiological studies do not provide any understanding of the evolution and histogenesis of pancreatic cancer. Experimental models of this disease have been developed during the past 12 years in a varety of rodent species, in order to evaluate the role of various risk factors and better understand the histogenesis and biological behaviour, and to be used as an effective system for various experimental manipulations aimed at preventing and altering the natural progresssion of pancreatic carcinoma. The major objective of this paper is to review different models of pancreatic carcinogenesis in rodents and to briefly discuss the histogenesis of these tumors.

Animals models of pancreatic neoplasms

Rats, 4-hydroxyaminoquinoline-1-oxide (4-HAQO)

4-HAQO, the presumed proximate carcinogen of 4-nitroquinoline-1-oxide, is both a mutagen and a carcinogen. A single intravenous injection of 4-HAQO at a dose of 6 to 10 mg/kg body weight produces atypical acinar cell foci (AACF) and acinar cell nodules [6–8]. AACF are subclassified, based on staining properties and cytoplasmic morphology, into basophilic foci (BF), acidophilic foci (AF), and acidophilic nodules (AN) [8–10]. The cells in the basophilic foci are large, with irregular nuclei. They contain deeply basophilic cytoplasm with a sparse number of zymogen granules, and are arranged as acini (Fig. 1). Ultrastructurally, the cells in BF have an abundant amount of rough endoplasmic reticulum (RER), with a decreased number of secretory granules (Fig. 2). The morphological features of cells in AF and AN are similar. The cells are arranged as acini and contain a basally located nucleus with a prominent nucleolus (Fig. 3). The cytoplasm is eosinophilic and coarsely granular. Ultrastructurally, these cells have a highly polarized pattern, with basally located nucleus and RER, and the zymogen granule-rich cytoplasm oriented in the opposite direction (Fig. 4). Both BF and AF showed decreased γ-glutamyltranspeptidase (GGT) activity in comparison to normal pancreas; however, the GGT activity is much less in BF than in AF.

Administration of a single injection of 4-HAQO at a dose of 6 mg/kg leads to the development of both BF and AF at as early as 6 wk, and these lesions progressively increase in number with time. The number of BF per pancreas increases from 10 ± 4 at 6 wk to 78 ± 8 at 24 wk. However, their volume increases only minimally from 6 wk ($107 \pm 16\,\mu$) to 24 wk ($198 \pm 6\,\mu$). This lack of growth is consistent with their decreased proliferative capacity [8]. AF not only increase in number (0.7 ± 0.3 at 6 wk and 35 ± 10 at 24 wk), but also markedly increase in volume ($105 \pm 55\,\mu$m at 6 wk and $495 \pm 46\,\mu$m at 24 wk) and become grossly visible. From their initial appearance, both BF and AF are morphologically distinct, and no transition from one type to the other is seen. With the single injection protocol, 100% of the animals develop

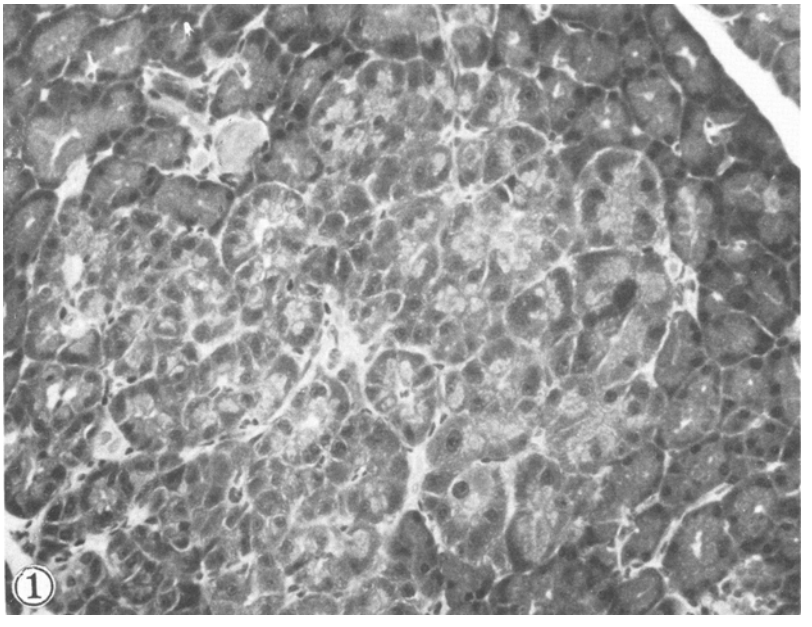

Fig. 1. Light micrograph of basophilic focus from a rat injected with a single dose of 4-HAQO, and sacrificed 25 weeks later. H & E stain, ×270.

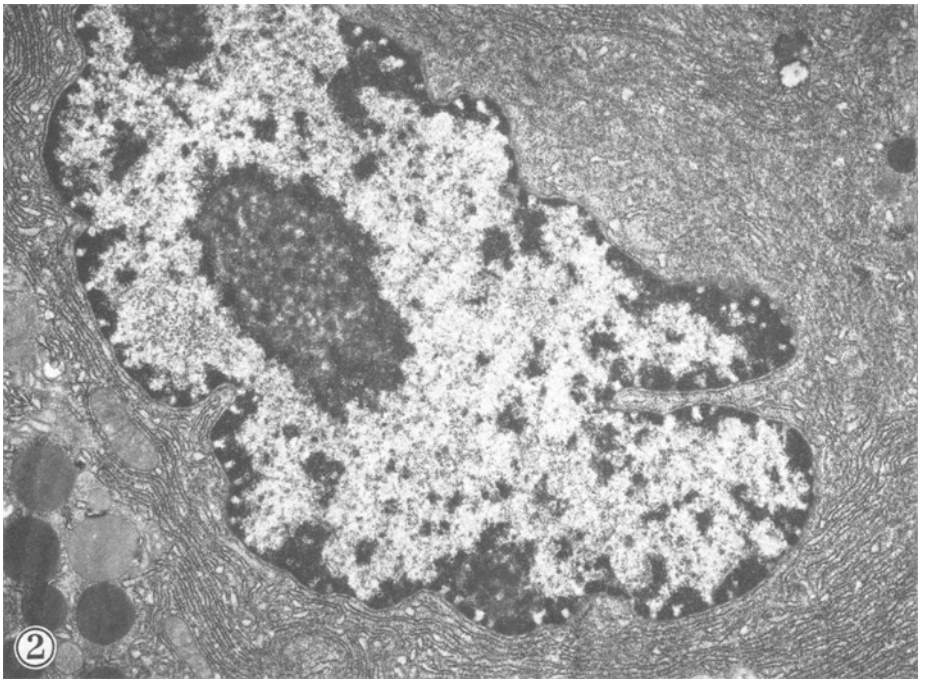

Fig. 2. Electron mirograph of a cell from basophilic focus showing irregular nucleus and abundant RER ×11,000.

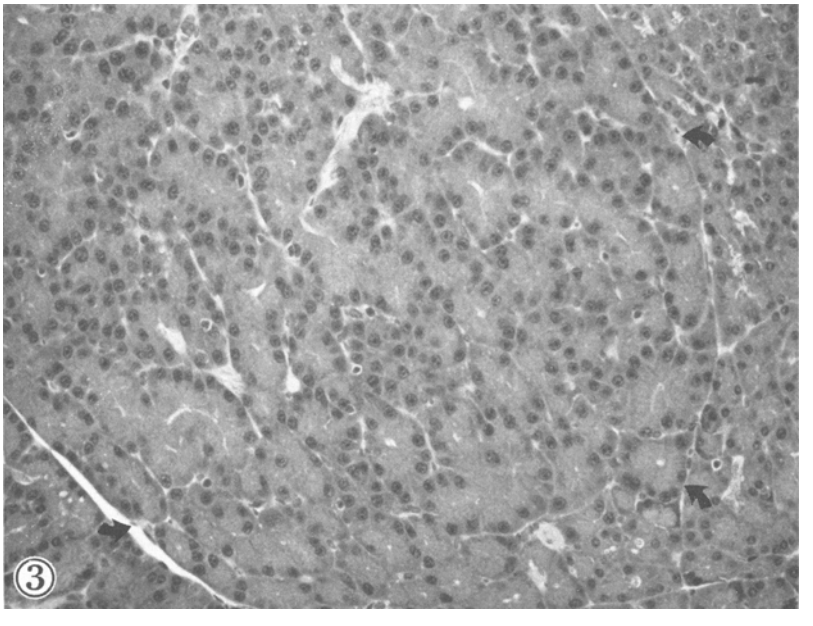

Fig. 3. Light micrograph of acidophilic focus (arrows) showing large acini with prominent nuclei, H & E stain, ×270.

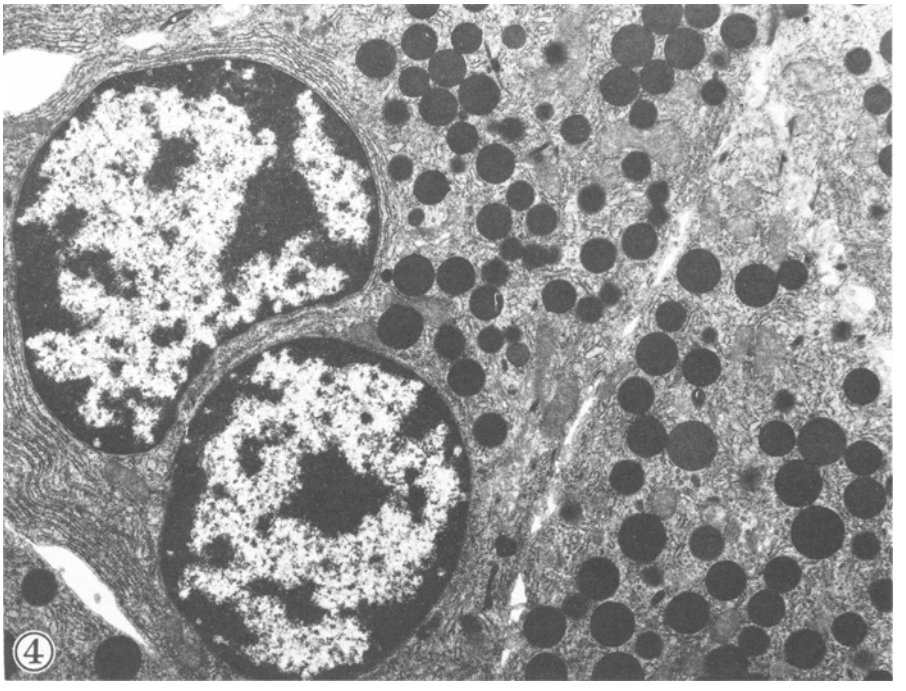

Fig. 4. Electron micrograph of cells from acidophilic focus showing basally located nuclei and large number of mature zymogen granules ×9600.

BF, AF, and AN, and only an occasional rat shows acinar cell carcinoma at the end of one year (Fig. 5). However, the carcinogenic potency of 4-HAQO can be markedly enhanced by administering it during maximal DNA synthesis in the pancreas. Konishi *et al.* [11] have induced acinar cell carcinoma of the pancreas in 60% of the rats following a single dose of 4-HAQO administered during peak DNA synthesis after partial pancreatectomy.

Azaserine

Azaserine (o-diazoacetyl-L-serine), an antimetabolite isolated from cultures of streptomyces, is a mutagen in Ames *Salmonella* typhimurium assay. It induces pancreatic DNA damage and inhibits DNA synthesis in the rat pancreas [12–14]. Single or multiple i.p. injections of azaserine at doses of 10 to 60 mg/kg produce atypical acinar cell nodules (AACN), adenomas, and adenocarcinomas [12, 15]. AACN appear as early as 1 month and carcinomas by 9 months after initial azaserine treatment [16, 17]. Longnecker and assocates have thoroughly described the morphological features of these various azaserine-induced acinar cell lesions [16, 17]. Azaserine-induced lesions are also of two distinct histological types and are classifiable as AF and BF [18]. Phenotypical alterations in the AACN and adenomas include decreased γ-glutamyltranspeptidase and reduced uptake of iron in animals overloaded with iron [19]. More than 70% of the adenocarcinomas induced by azaserine are well to poorly differentiated acinar cell carcinomas, and the remaining are of mixed pattern, showing acinar cells and other cell types [16, 20]. Many of these tumors metastasize to liver, lymph nodes, and lungs [12, 16]. Azaserine also induces tumors at several other sites, including kidney, breast, and skin [12].

7,12-Dimethylbenz(a)anthracene (DMBA)

Dissin *et al.* [21] have induced pancreatic carcinoma after implanting 2 to 3 mg of crystalline DMBA,

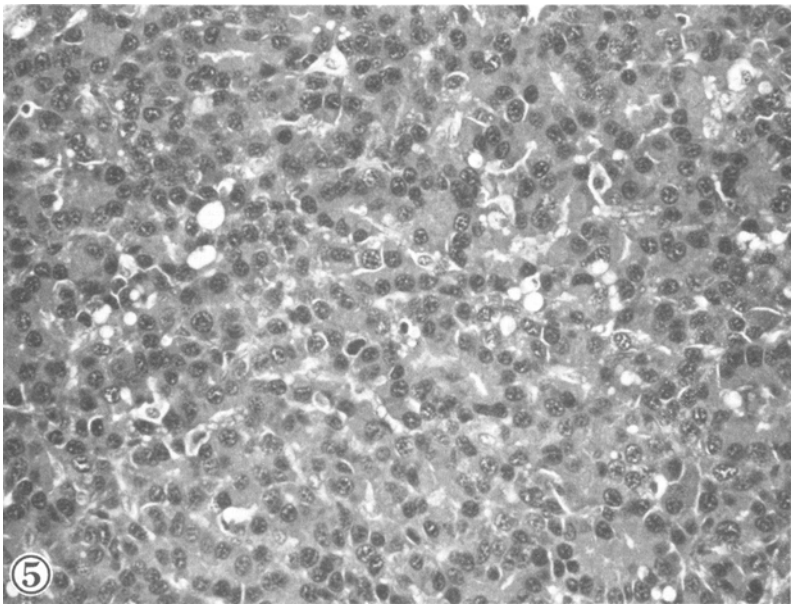

Fig. 5. Acinar cell carcinoma of pancreas induced by a single dose of 4-HAQO. H & E stain, ×200.

a polycyclic hydrocarbon, in the head of the pancreas. About 72% of the rats develop tumors between 119 and 363 days after implantation. Histologically, these tumors show features of poorly differentiated adenocarcinomas, but acinar cell features have been identified on electron microscopy [22]. Most of the induced tumors in this model are malignant, and have metastasized into the peritoneal cavity. In addition to the malignant tumors, development of tubular complexes and adenomas has also been reported.

Miscellaneous models

Hypolipidemic compounds

Prolonged administration of peroxisome proliferators to rats and mice results in the development of hepatocellular carcinomas [23, 24]. Some of these agents also induce pancreatic tumors. Reddy and Rao [25] reported a 20% incidence of pancreatic tumors in F-344 rats fed nafenopin (0.1% w/w) in diet. These tumors included two adenomas and one metastasizing acinar cell carcinoma. The acinar cell carcinoma is being maintained as a transplantable tumor in syngeneic rats [9, 26]. With two other hypolipidemic peroxisome proliferators, the development of AF, adenomas, and acinar cell carcinomas has also been reported [27, 28].

N-(N-methyl-N-nitrosocarbamoyl)-L-ornithine (MNCO)

MNCO is a nitrosourea amino acid, a direct acting carcinogen that has specific affinity for kidney and pancreas [17, 29]. Low doses of MNCO lead to the development only of AACN, whereas higher doses result in the development of AACN, adenomas, and acinar cell carcinomas [30]. Histological features of the MNCO-induced pancreatic lesions are similar to those induced by azaserine [16]. In addition to the pancreas, MNCO also induces tumors in kidney, breast, ear duct, and skin.

Corn oil

In a recent study Eustis and Boorman [31] reported

a high incidence of focal acinar hyperplasia and acinar adenomas in rats given corn oil by gavage for 2 yr as compared to the control rats. However, it is not clear whether this increased incidence is due to the promoting effect of corn oil on spontaneously induced acinar cells, or to the carcinogenic effect of corn oil itself.

Nitrosamines

Di-n-propylnitrosamine and its β-oxidized derivatives N-nitrosobis(2-oxopropyl)amine (BOP) and N-nitroso-(2-hydroxypropyl)(2-oxopropyl)amine (HPOP) are potent pancreatic carcinogens in hamsters [32–34]. In rats a single injection of BOP (100 mg/kg) or HPOP (20 mg/kg) induces AACN in 4 months [35]. Injection of a higher dose of HPOP (160 mg/kg) results in the development of AACN, adenomas, and acinar cell carcinomas [36]. Unlike hamsters, no ductal tumors are induced in rats.

Guinea Pig

In 1968 Drückery *et al.* [37] showed that the prolonged administration of methylnitrosourethan or methylnitrosourea (MNU) in drinking water to random bred guinea pigs produced adenocarcinomas of the pancreas in 25% of the animals in between 740 to 800 days. This model was improved by Reddy and associates [38, 39], who gave freshly dissolved MNU once a week to inbred NIH strain 13 guinea pigs. With this approach, tumors developed in 29% of the animals in between 28 to 44 weeks. Histologically, these tumors showed varying degrees of adenocarcinomatous differentiation (Fig. 6). Non-tumorous portions of the pancreas showed ductular or pseudoductular transformation of the acini (Fig. 7).

Hamster

After the initial description of the induction of pancreatic tumors with diisopropylnitrosamine in hamsters by Krüger *et al.* [40] and Pour *et al.* [41], several of its oxidized derivatives, such as BOP, HPOP, N-nitrosobis(2-acetoxypropyl)amine, N-nitrosomethyl(2-oxopropyl)amine (MOP), and N-nitroso-2,6-dimethylmorpholine, have been identified as potent pancreatic carcinogens [42–47]. Interestingly, all of these chemicals induce ductal adenocarcinomas that closely resemble the most common malignant pancreatic neoplasms in humans. However, the carcinogenic potency of these compounds varies considerably, with BOP and MOP being the most potent [42, 45]. The other organs or tissues in which these compounds induce tumors include lungs, trachea, larynx, nasal cavities, liver, gall bladder, kidneys, salivary glands, and blood vessels. Furthermore, the organotropism of these compounds also varies with the route of administration. Local implantation or oral administration are less effective in inducing pancreatic tumors than subcutaneous injection [48, 49].

BOP is not only the most potent carcinogen of this group of nitrosamines, but is also the most pancreas-specific. A single or multiple injection of BOP induces a high incidence of pancreatic adenomas, *in situ* carcinomas, and invasive carcinomas of ductal origin in 80% to 100% of the animals in between 13 to 50 wk [42, 50, 51]. Histologically, the majority of tumors are well differentiated tubular adenocarcinomas (Fig. 8). A small percentage of the tumors are poorly differentiated and show excessive production of mucin, papillary pattern, or adenosquamous features. The adenocarcinomas invade locally into the peritoneal cavity and metastasize to the regional lymph nodes and lungs. Some of the BOP-induced pancreatic adenocarcinomas are easily transplantable into nude mice and syngeneic hamsters [52, 53]. One transplantable tumor maintained by Scarpelli and Rao [52] has been converted into an ascitic form and has also been maintained in tissue culture as a cell line, and is being used to study the effect of chemotherapeutic agents [54, 55].

Mouse

The mouse has very rarely been used in experimental pancreatic carcinogenesis. Roebuck and

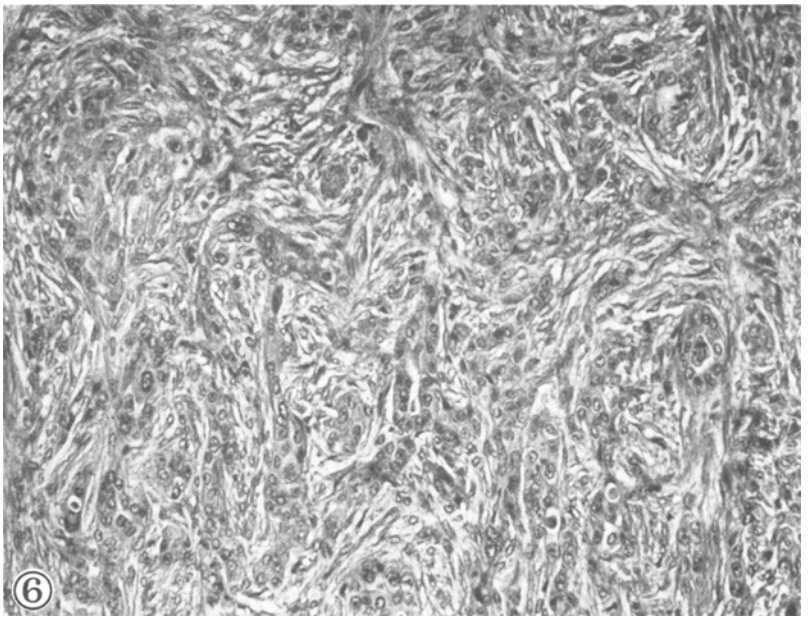

Fig. 6. Infiltrating adenocarcinoma of pancreas in a guinea pig treated with MNU. Marked desmoplastic reaction is seen. H & E stain, ×330.

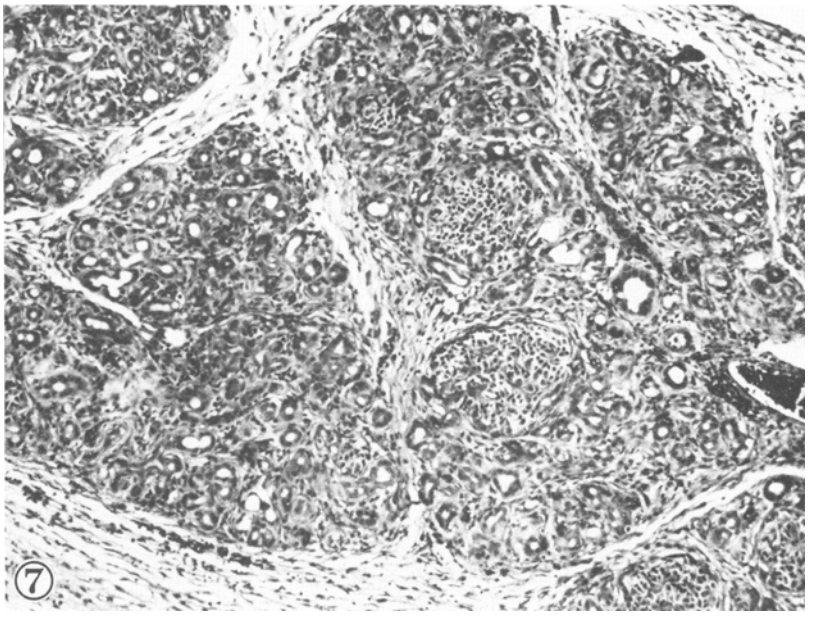

Fig. 7. Pseudoductular change in the pancreas of a guinea pig treated with MNU. H & E stain, ×180.

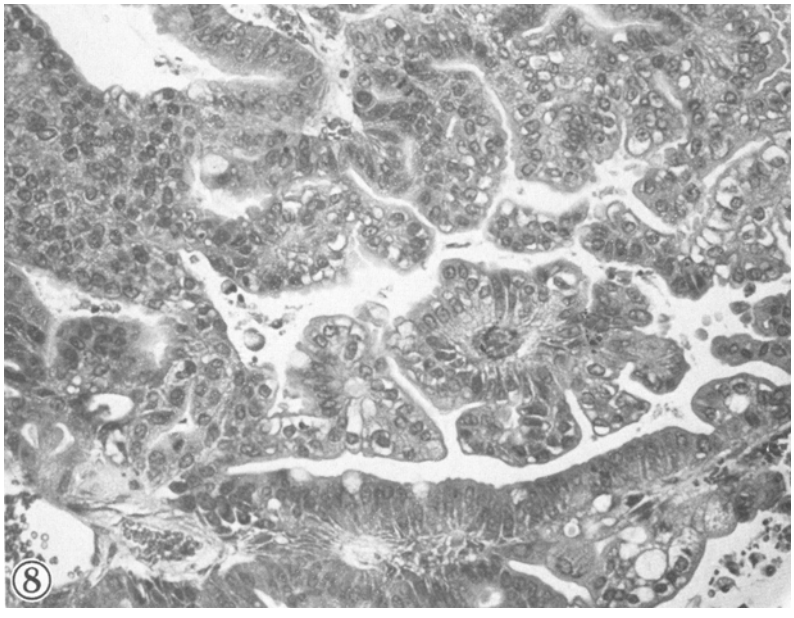

Fig. 8. Well-differentiated ductal adenocarcinoma of the pancreas from a hamster treated with multiple doses of BOP. H & E stain, ×220.

Longnecker [56] reported the development of AACN in mice given azaserine. A single i.p. injection of MNU in month old mice induced acinar cell carcinomas in 18% of the animals [57]. We have recently shown that a single i.v. injection of 4-HAQO at a dose of 24 mg/kg body weight in 5–6 wk old Swiss Webster mice induces AACF in 100% of the animals [58]. Interestingly, unlike rats, mice develop only acidophilic foci with increased mitotic activity and labeling indices. The AF showed decreased GGT activity. No basophilic foci are observed.

Histogenesis of pancreatic tumors

Acinar cells are the major cell type in the exocrine pancreas and constitute about 82% of the total volume, whereas duct cells comprise only 3.9% [59]. Surprisingly in humans, however, the majority of the exocrine pancreatic carcinomas are classified as ductal adenocarcinomas. This histogenetic classification is based mainly on the similarities of carcinoma cells to ductal cells by light microscopy, by their ability to produce mucins and the associated ductal changes such as hyperplasia, dysplasia, and carcinoma *in situ* [60–62]. Acinar cell carcinomas are considered to be relatively rare and may account for 10% of the total carcinomas [63]. This low percentage may not be a reflection of the true incidence of acinar cell carcinomas, since most of the tumors are classified solely by histology, rather than by ultrastructural features and functional markers. In this context it is interesting to point out that most of the solid and papillary epithelial neoplasms of the pancreas show acinar cell features on ultrastructural and immunocytochemical examination [64]. At present, although the majority of human pancreatic carcinomas are believed to arise from duct cells, the exact histogenesis remains unclear and controversial.

The histogenesis of these tumors in animal models of pancreatic carcinogenesis is equally ambiguous. The basic arguments in this regard include the question of whether the tumors arise purely from duct cells, from dedifferentiated acinar cells, or from both cell types. The concept of acinar cell dedifferentiation has not been well accepted because it contradicts the dogma concerning the embryologic development of pancreas, in which islet

and acinar cells develop from the duct system. Recently, however, it has been clearly shown that under various experimental manipulations, such as simple trauma to pancreas or injection of pancreaticotoxic chemicals, the acinar cells can be transformed into pseudoductules or even well-differentiated hepatocytes [65–68]. It is pertinent to note that when dissociated acinar cells of moderately differentiated acinar cell carcinoma are maintained *in vitro* on basement membrane, they show ductular arrangeent [69].

In the rat model the pancreatic lesions induced by several carcinogens and corn oil retain fully differentiated acinar cell features, except with DMBA. No pseudoductular or dedifferentiated features are present. However, the development of carcinomas induced by local implantation of DMBA in the pancreas is preceded by the formation of tubular complexes that are considered precursor lesions [21, 22]. Wax reconstruction studies and ultrastructural analysis reveal acinar cell features in the cells lining tubular complexes and carcinomas [22, 70]. Similary, MNU-induced pseudoductular lesions in the pancreas of guinea pigs also show features of acinar cell dedifferentiation [71].

Histological examination of fully developed carcinomas of the pancreas induced by various β-oxidized derivatives of dipropylnitrosamine reveals ductal/ductular features. Based on this information, Pour [72] proposes that all pancreatic carcinomas are derived from ductal or ductular cells. Interestingly, sequential analysis of pancreases of BOP- and BHP-treated hamsters show the earliest changes in acinar cells, characterized by the formation of pseudoductules and cystic complexes [51, 73, 74]. Based on these findings, Scarpelli *et al.* have postulated that pancreatic tumors in hamsters develop from both the ductal cells and dedifferentiated acinar cells [51].

Conclusions

The pathogenesis of pancreatic tumors is complex, and depends upon the species rather than on the type of carcinogen administered. In rats, various types of structurally unrelated carcinogens administered under different experimental conditions induce only acinar cell lesions, whereas in hamsters the majority of tumors appear to arise from duct/ductular cells, and the remaining from dedifferentiated acinar cells. It is not clear why acinar cells in the rat are so susceptible to the carcinogenic effect and duct cells are so sensitive in the hamster. This difference may be related to the quantitative and qualitative differences in the drug metabolizing enzymes present in the acinar and duct cells, which activate procarcinogens to ultimate carcinogens. Biochemical, autoradiographic, and immunohistochemical stains have been used to show that acinar cells of both rats and hamsters contain drug metabolizing enzymes [75–78]. However, significant differences are noted in the content of these enzymes in the duct cells of rat and hamster pancreas. Baron and Kawabata [79] have shown that the levels of some of the isozymes of cytochrome P-450 in pancreatic duct epithelial cells of rat are very low, whereas in hamster the levels are comparable to acinar cells.

The other highly interesting fact in the histogenesis of pancreatic adenocarcinoma is the role of acinar cells. The conversion of acinar cells to ductular complexes and the transdifferentiation to hepatocytes attests to their plasticity. The carcinogen-induced tubular complexes may serve as a precursor lesion for the development of carcinoma. The role of dedifferentiation of acinar cells in the development of carcinoma is supported by the observation of the expression of fetal acinar cell antigens in carcinomas [80]. The significance of the histogenesis of pancreatic tumors becomes relevant and important if there is a difference in the progression and biological behaviour of the tumors that arise from ducts or dedifferentiated acinar cells. In this connection it is pertinent to point out that foci, atypical acinar cell nodules, and ductular complexes have also been observed in human pancreases [81]. However, it is not yet clear whether these represent preneoplastic lesions.

Key unanswered questions

- Are there any stem cells in the pancreas from

which some tumors arise?
- Is there a direct approach to proving that acinar cells can indeed undergo retrodifferentiation?
- Are there any differences in the biologic behaviour of the tumors that arise from duct cells and dedifferentiated acinar cells?

Acknowledgements

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