Interface between adaptive and neoplastic growth in the pancreas

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SUMMARY The adaptive changes of hypertrophy and hyperplasia are diffuse and reversible responses of the pancreas to growth promoting stimuli. Early stages of neoplastic growth in the pancreas have been studied in carcinogen treated animals and preneoplastic lesions including atypical acinar cell foci and nodules, tubular ductal complexes and intraductal hyperplasia were identified. Neoplastic growth is clonal rather than diffuse and involves multiple steps through preneoplastic stages to produce a tumour. The individual steps are commonly regarded as reflecting a series of changes in the genome of the cells. Although the changes are likely to be irreversible, completion of the sequence usually requires a major portion of the lifespan of the host. The rate of progression of preneoplastic lesions to cancer may be modulated by the same factors that control adaptive growth. It follows that such factors will influence the probability that a carcinoma will develop. Cholecystokinin (CCK) seems to provide one example of a hormone/growth factor that can stimulate normal, adaptive, and neoplastic growth, and it is to be expected that other such hormones will be identified.

Examination of the conceptual interface between adaptive and neoplastic growth in the pancreas implies a need first to consider normal adaptive growth and, second, the early phases of neoplastic transformation and growth. The former topic will be dealt with selectively to highlight aspects of normal growth that have special relevance for carcinogenesis. In the context of carcinogenesis in the exocrine pancreas, two types of cells must be considered – acinar cells and ductal cells.

NORMAL AND ADAPTIVE GROWTH

Although all exocrine cells are regarded as derivatives of common ancestors that arise as an evagination of the primitive intestinal tract midway during embryonic development, it is accepted that both cell types can divide following differentiation.[1] This appears to be true late in embryonic growth and also in the adult animal during pancreatic regeneration. The pancreas grows rapidly during the early postnatal period as oral feeding begins.[2][3] It seems likely that this postnatal growth spurt in the pancreas is mediated by CCK, and perhaps by other gastrointestinal peptide hormones.[3][4] Both acinar cells and ductal cells must grow during this period.

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Oates and Morgan have reported that 95% of acinar cells are mononucleated during the early postnatal period in rats whereas in adult animals this fraction falls to below 40%.[3] Fractions in the range of 60–80% of acinar cells have been reported to be binucleate in rats older than eight weeks.[3][5] It seems likely that actively cycling acinar cells are mononuclear even in adult animals. The overall picture is consistent with the possibility that binucleation reflects terminal differentiation in acinar cells and that the mononucleated acinar cells function as a reserve cell population and might be regarded as 'stem cells'.

Whether there are both terminally differentiated and stem cell pools in the ductal epithelium is not known. Reports that islet cells may be formed in the walls of ducts in adult animals suggest that at least some duct cells can function as 'stem cells'.

Hypertrophy (increase in cell size) and hyperplasia (increase in cell number) are normal responses of cells to physiologic growth stimuli. In the pancreas, such a stimulus appears to be provided by CCK. One expects virtually all acinar cells to be enlarged in the hypertrophic pancreas, and mitoses to be widely scattered in the acinar and ductal cells during hyperplasia. Hypertrophy and hyperplasia are diffuse responses of cells throughout the pancreas.

Adaptive change within cells may also involve metaplasia (a change in differentiation from one adult cell type to another). Metaplasia of ductal glandular epithelium to squamous epithelium has been described in the pancreas. All adaptive changes are considered to be reversible if the evocative stimulus is withdrawn.

NEOPLASTIC GROWTH

A key concept in distinguishing neoplastic growth from normal growth is that the former reflects altered growth of single cells – that is, clonal growth and expansion. Multiple microscopic foci of abnormal cells may develop in the pancreas of a carcinogen exposed animal, but the change is not diffuse. Clones with high growth potential continue to expand to become grossly visible masses and perhaps ultimately will develop into neoplasms. The sequence may be represented as follows: initiated cell→focus→ nodule→neoplasm (carcinoma).

Examination of the interface between normal and neoplastic growth requires recognition of focal abnormalities of growth at an early stage of their development, recognition of the cell of origin of the abnormal clone, distinction between lesions of different types in regard to their potential for progression to malignancy, and recognition of the effect of normal growth stimuli on the formation and growth of preneoplastic clones. Two aspects of carcinogenesis are important in considering this interface. First, dividing cell populations are more likely to sustain and express mutations that may lead to malignancy than are non-dividing cells. Rats are highly sensitive to the induction of preneoplastic lesions when carcinogens are given during the first three weeks of life or during pancreatic regeneration.[26] Second, growth factors (hormones) that stimulate normal cells to divide may also stimulate the growth of initiated cells, thus speeding the completion of the carcinogenesis sequence.

Abnormal clones are recognised in tissue sections by phenotypic changes including an increased rate of cell division, altered enzyme content of the cells, changes in nuclear size, or changes in differentiation. Loss of differentiation is one of the most apparent histologic hallmarks of neoplastic transformation. Dysplasia and anaplasia are terms often used to denote mild and severe degrees of failure to differentiate normally, and the latter has the connotation of malignancy. In some cases the phenotypic change in carcinogen induced lesions is extreme and constitutes metaplasia to a different cell type.

Another concept that is essential for discussion of the change from normal to neoplastic growth is that the transition appears to be stepwise in most clones as has been emphasised for hepatic neoplasia.[7] A

suggestion of such steps can sometimes be seen by the presence of a secondary phenotypic change within a carcinogen induced lesion. Such changes have been called 'nodules within nodules' in the liver and the secondary change is often marked by a further loss of differentiation – that is, a dysplastic or anaplastic change. It is probable that each of these 'steps' reflects a mutation or activation of a cellular oncogene by some other mechanism.

Given this conceptual base for examination of the earliest stages of carcinogenesis, experimental observations in the pancreas can be considered. More than 20 chemicals have been shown to induce pancreatic carcinoma in experimental animals.[8-10] Most of these chemicals are N-nitroso compounds, and the majority require metabolism to become active carcinogens. The pancreas contains low levels of the cytochrome P-450 enzymes that mediate the metabolism of xenobiotics, and the pancreas appears to be capable of activating at least some carcinogens.[10] Several of these chemical carcinogens have been used to establish animal models of pancreatic carcinogenesis in which the development of cancers has been carefully studied. The best characterised models are in rat and hamster. The preneoplastic and neoplastic lesions that develop in rats and hamsters differ considerably. In rats, the carcinogens seem primarily to affect the acinar cells, the majority of pancreatic carcinomas seem to originate in these cells and most are acinar cell carcinomas. In hamsters, both acinar cells and ductal cells are affected by the carcinogens and it appears that cancers arise from both cell types. Most pancreatic carcinomas in hamsters, however, have a ductlike histologic appearance. Several different preneoplastic lesions that have been identified in carcinogen treated rats and hamsters have also been found in the human pancreas suggesting that the same pathways of tumour development occur in humans and animals.

Examples of carcinogen induced focal lesions that have been described in the pancreas of experimental animals are listed in the Table. This list has been kept relatively simple. Terminology is not standardised and the Table lists the more common alternate names for the lesions. Several reviews provide more detailed discussion of these terms.[8-12]

The atypical acinar cell focus is most characteristically found in the pancreas of carcinogen treated rats, although similar lesions have been described in mice, hamsters, guinea pigs, and man. These lesions are a heterogeneous group and the phenotypic change that allows their recognition includes features which may occur alone or in combination. Focal growth of highly differentiated acinar cells may represent the minimal change in the category, but a recent report indicates that even the most highly differentiated

Table *Summary of terms used to designate carcinogen-induced histologic changes in the pancreas.*

Term with subtypes	Alternate terms
Atypical acinar cell focus (or nodule) acidophilic focus*	Hyperplastic acinar cell nodule Focal acinar cell dysplasia Focal acinar hyperplasia Acinar cell nodule Acinar adenoma
basophilic focus	Basophilic cellular change Hypertrophic focus
Hepatocyte-like focus	Eosinophilic cell metaplasia
Cystic ductal complex	Cystic duct complex Cystic-fibrotic lesion Cystadenoma
Tubular ductal complex*	Pseudoductular transformation Tubular complex Ductular hyperplasia
Intraductal hyperplasia* papillary atypical	

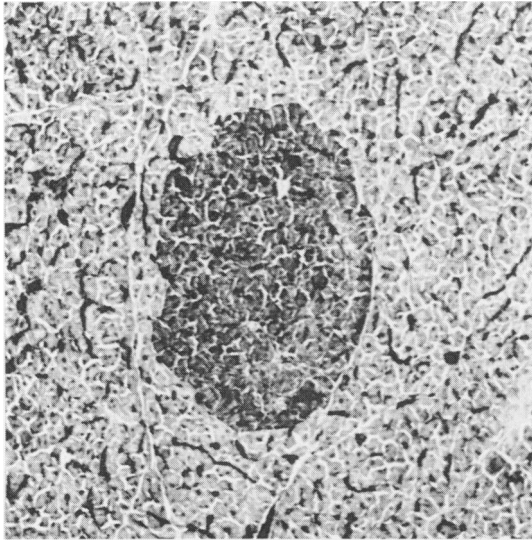* marks lesions regarded at highest risk for progression to carcinoma.

Fig. 1 *ATPase-positive atypical acinar cell focus in the pancreas of a rat killed four months after carcinogen (azaserine) treatment. Courtesy of R. Woutersen [13]. ATPase stain. × 120.*

acinar cell foci have changes in enzyme content that can be recognised by an appropriate histochemical stain – for example ATPase (Fig 1).[13] Another interesting observation is that the majority of the cells of acinar cell nodules are mononucleated.[5] This appears to be a relatively early change as it was present in the smallest nodules studied, and suggests that the nodules are composed of 'stem' acinar cells. It was not possible to isolate foci smaller than 1 mm³

so it is not known if the earliest lesions are composed of mononucleated cells, although this seems probable.

Some acinar cell foci – for example, a subcategory classified as basophilic or hypertrophic foci,[12] seem to have no or only low potential for growth and progression to neoplasms, whereas a higher fraction of lesions that are classed as acidophilic seem to have potential for such progression.[14] Overall, it appears that fewer than 1% of acinar cell foci have the potential to progress to malignancy. As lesions become larger or show anaplasia as a secondary phenotypic change (Fig 2), the probability of progression to malignancy increases greatly.

Although it has not been shown directly that CCK stimulates the growth of atypical acinar cell foci, this may be inferred from the observation that feeding diets that contain trypsin inhibitors promote the growth of such foci and the development of carcinomas in azaserine treated rats.[4]

Ductal complexes also comprise a heterogeneous group (Fig 3) that are found in both hamsters and rats. There is a growing acceptance of the origin of lesions of this type from acinar tissue as a result of metaplastic change of acinar cells to ductlike cells.[15-17] The original view for the origin of such lesions is that they result from the proliferation of intralobular ductal cells.[11] It is not clear what fraction of lesions arise by one or the other of these mechanisms. Acinar cells are sometimes found in the lining epithelium of both cystic and tubular lesions.

We have recently found that a higher fraction of ductal complexes of intermediate histologic type – that is, those lined predominantly by cuboidal cells, contain acinar cells than is found in either cystic or tubular ductal complexes in hamsters four months
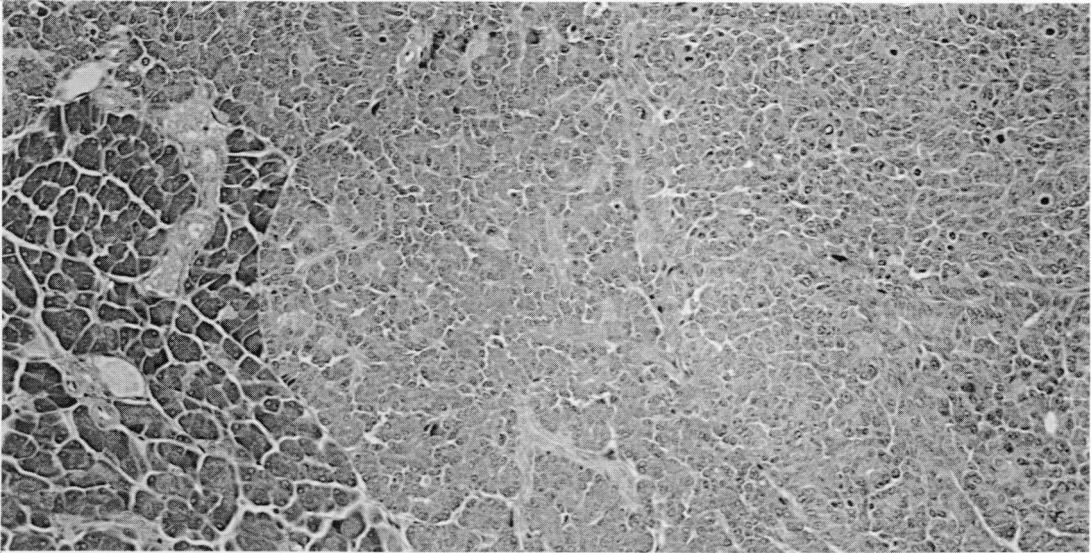
Fig. 2   *Large atypical acinar cell nodule with secondary phenotypic change (anaplasia) from the pancreas of a rat that was treated with five azaserine injections ending one year before autopsy. Normal pancreas, lower left, primary nodular cells, centre, and secondary nodule, right. All have different appearances. Haematoxylin and eosin. × 170.*
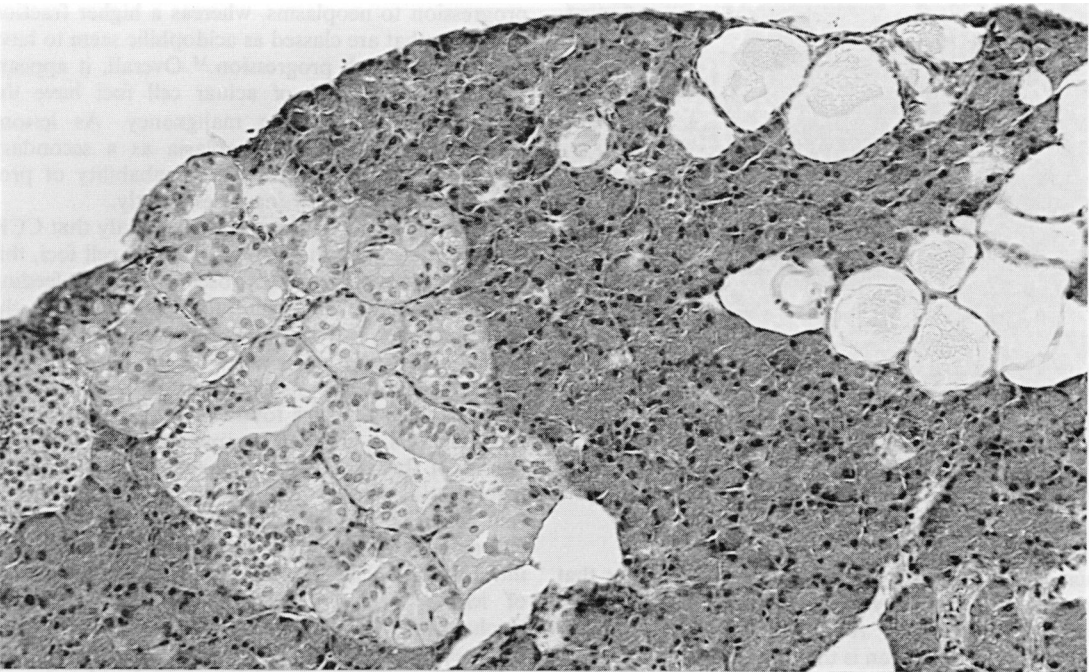

Fig. 3   *Pancreas from a hamster treated four months earlier with three weekly injections of N-nitrosobis(2-oxopropyl)amine.*[18] *Two cystic ductal complexes (right) and a tubular ductal complex (left) replace acinar tissue in the periphery of a lobule. An islet lies at the left border. Cystic spaces are lined by flattened epithelium in the cystic ductal complex, and the ratio of lumen diameter to epithelial cell height is high. In the tubular ductal complex, the tubules are lined by cuboidal or columnar cells. The ratio of lumen diameter to epithelial height is low. Haematoxylin and eosin. × 170*
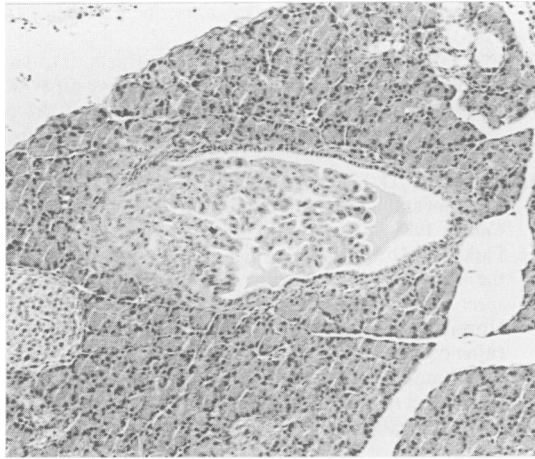
Fig. 4 *Intraductal hyperplasia in the pancreas of a hamster that was treated with BOP four months earlier. The epithelial overgrowth can be seen to involve only part of the lining epithelium suggesting that the hyperplastic lesion had a clonal origin. Haematoxylin and eosin.* × *100.*

after carcinogen treatment.[18] We have speculated that such lesions might represent a first step in the formation of cystic and tubular ductal complexes. In lesions with lumenal spaces lined by cells with low proliferative potential there would follow a progressive flattening of surviving epithelial cells so that cystic spaces would be lined by low cuboidal or simple squamous epithelium, yielding the cystic ductal complex. In our experience cystic ductal complexes seldom achieve a diameter larger than 1 mm, and no carcinomas of similar histologic appearance have been observed. Larger lesions of this type have often been classified as cystadenomas.[11]

If a ductal complex contained one or more clones of epithelial cells with high proliferative potential, a lining epithelium of cuboidal or columnar cells would be maintained in the tubules yielding the appearance of a tubular ductal complex. It follows that the tubular lesions would have a greater capacity to grow to large size and a higher probability of completing additional steps for progression to carcinoma because of the higher rate of cell division. The histologic appearance of tubular ductal complexes is similar to the ductlike carcinomas that characteristically develop in hamsters treated with pancreatic carcinogens. A recent report suggests that the formation, growth or progression of tubular ductal complexes to carcinomas can be stimulated by injections of CCK.[16]

The development of hepatocyte like foci within the pancreas of carcinogen treated hamsters and rats has been characterised in the hamster by Rao *et al*,[6] and seems to be a florid metaplastic change of acinar or ductal cells that reflects the close embryonic relationship of the pancreas and liver. Hepatocyte like foci seem to have low growth potential and no carcinoma of this cell type has been described in hamster pancreas.

Intraductal hyperplasia appears to reflect clonal growth of ductal cells lining pancreatic ducts of any size category including interlobular ducts (Fig. 4). This change has been seen in carcinogen treated hamsters but not in rats. Epithelial hyperplasia may be accompanied by metaplastic change, and increased mucus production is common. The lesions with high growth potential may develop a pseudostratified columnar epithelium, a multilayered epithelium, a papillary pattern, or secondary anaplastic changes. The histologic features of these lesions overlap the spectrum of carcinomas that develop in hamsters.

In view of the growing evidence that pancreatic carcinogenesis in both rats and hamsters is stimulated by raised plasma CCK concentrations, the question arises as to a possible role for CCK in the genesis of human carcinomas. Two reports provide indirect support for this hypothesis. Plasma CCK concentrations have been reported to reach higher levels after oral administration of fat in patients with subtotal gastrectomy than in normal controls.[19] Subsequently, a strong association was found between pancreas cancer and a history of subtotal gastrectomy in an epidemiologic study.[20] These two independent observations fit nicely together to suggest that repeated episodes of excess stimulation of the pancreas by CCK may promote or serve a cocarcinogenic role for carcinogenesis in the human pancreas.

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