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Biology of pancreatic cancer

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Abstract

Pancreatic cancer is the fifth leading cause of death from malignant disease in Western society. Apart from the fortunate few patients who present with a resectable small pancreatic adenocarcinoma, conventional treatment offers no hope of cure and has little palliative value. Over the past two decades major steps have been made in our understanding of the biology of pancreatic growth and neoplasia. This review sets out to explore these advances, firstly in the regulation of normal pancreatic growth, and secondly the mechanism which may be involved in malignant change of the exocrine pancreas. From an understanding of this new biology, new treatment strategies may be possible for patients with pancreatic cancer.

Carcinoma of the exocrine pancreas is now the fifth leading cause of death from malignant disease in Western society[1] yet there has been only marginal improvement in the outcome of treatment for pancreatic cancer since the beginning of this century.[2] This improvement is largely due to the use of radical surgery with low mortality in specialist centres of excellence.[3] The diagnosis of carcinoma of the pancreas still remains a virtual death sentence for the patient. Unfortunately, most studies show an increasing incidence of the disease, particularly among the elderly, in whom it seems to have trebled over the past 50 years.[1] It is also apparent that despite advances in surgical technique, anaesthetic support, intensive care, anticancer chemotherapy, and radiotherapy, the five year survival rate for all patients diagnosed as having carcinoma of the pancreas is less than 1%.[2] The only cure at present for pancreatic cancer is surgical resection, which in the very few patients who present with tumours smaller than 2 cm in diameter may achieve five year survival rates as high as 37%.[4] It seems clear therefore that if any improvement is to be made in the treatment of this awful condition then a radical rethink is necessary in approaches to treatment and this must begin with a thorough understanding of the biology of the disease from which potential new remedies might be designed. The purpose of this review is to set out our current knowledge of the biology of pancreatic cancer and explore some of the potential avenues of treatment which may arise.

Cancer is an abnormality of cell division and tissue growth. In necropsy studies ductal hyperplasia, which may be premalignant, and separate synchronous carcinoma in situ can be found in as many as 20% of patients who die from pancreatic cancer.[5] Allen-Mersh[6] carefully dissected 102 pancreases taken at necropsy from 102 patients dying from non-pancreatic disease and found evidence of ductal mucinous hyperplasia in 63 separate pancreases. In both the established carcinogen induced rodent models of pancreatic cancer, azaserine treated rats which develop acinar tumours[7] and *N*-nitrosobis(2-oxopropyl) amine treated hamsters which causes ductal disease[8 9] similar to most human disease,[10] malignant tumours are preceded by hyperplastic and nodular changes and also an alteration of pancreatic endocrine activity.[11] It seems rational therefore to start with an overview of the regulation of normal pancreatic growth, discussing the possible abnormalities in this process which may lead to or promote tumour growth.

Normal pancreatic growth

Pancreatic development in humans first becomes detectable as two separate evaginations of the primitive foregut during the fifth week in utero. Over the subsequent two weeks these dorsal and ventral buds fuse to form a single glandular structure from which develop the ductular and acinar components of the exocrine pancreas and the separate cellular components of the endocrine pancreas.[12] Specific cell types arise either by mitotic division of existing cells, differentiation of specialised cells, such as ductal cells, from uncommitted precursor or stem cells, or by transformation of one type of differentiated cell to another type – for example, acinar to ductal.[13] Despite the evidence that a major attack of acute pancreatitis in humans which results in loss of acinar tissue, if survived, will be followed by full recovery of both structure and function of the exocrine gland,[14] no other objective data are available on the regulation of exocrine pancreatic morphology in humans. Most of our data are derived from animal studies, usually in rats.[15]

During fetal life cell replication occurs before acinar cell differentiation[16]; however, there is a high frequency of mitoses in fully differentiated acinar and ductal cells which exists even late in the gestational period[17] and values as high as 14% have been reported for acinar cells immediately before birth.[18] Over the period of suckling, however, this labelling index rapidly decreases to levels of 1%, falling one month postnatally to 0·1–0·2%, where it remains thereafter.[18] Indeed, with advancing age there is even a loss of the normal pancreatic trophic response to hormones (cholecystokinin, secretin, bombesin, penta-

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gastrin) which normally promote pancreatic growth.[19 20]

The mature pancreas will undergo hyperplastic growth in response to three situations: dietary manipulation, surgical procedures, and hormone stimulation. Obviously the result of each of these events does not occur in isolation from the other two but the evidence for each is best described individually.

Diet

Starvation in the rat will cause an appreciable reduction in total pancreatic DNA content after six days.[21] These changes can be prevented by administering pentagastrin[22] and during starvation are associated with a commensurate fall in intestinal mucosal cholecystokinin content and a compensatory increase in intestinal cholecystokinin content during refeeding which coincides with the recovery of pancreatic DNA content.[22] In a subsequent study the same researchers showed that chronic feeding with cholestyramine, which binds luminal bile salts, produced pancreatic hyperplasia.[23] Similarly, inhibition of the other major components of pancreatobiliary secretion, the pancreatic enzymes, and in particular trypsin, by soya bean trypsin inhibitor, promotes pancreatic growth.[24 25] Diet also modulates the response of the pancreas to epidermal growth factor. A diet in which two thirds of the calories are administered as fat causes increased amino acid incorporation into rat acinar cells in response to epidermal growth factor stimulation.[26]

Surgical procedures

In 1885 Di Mattei reported that pancreatic resection in the dog resulted in pronounced mitotic activity in the residual gland.[27] More recent studies have shown that as little as 50% pancreatectomy will promote pancreatic regeneration in the remaining tissue in the rat with maximal incorporation of tritium labelled thymidine into replicating acinar cells seen as early as 36 hours after resection.[28] Surgical pancreaticobiliary diversion of bile and pancreatic juice away from the proximal small bowel promotes pancreatic growth,[29] and vagal deafferentation after jejunectomy causes pancreatic atrophy in the pig,[30] suggesting a feedback role of pancreatic secretion in maintaining pancreatic size.

Ileocaecal resection[31] and colectomy[32] both promote pancreatic growth in the rat and several studies have shown that this response is not due to the subsequent hypergastrinaemia associated with these procedures.[33-35]

Hormones and other growth factors

It became apparent in the early 1970s that gastrointestinal hormones were of fundamental importance in the maintenance of gut integrity and promotion of intestinal growth. By the mid 1970s Enochs and Johnson were proposing that cholecystokinin, which is a prime mover of pancreatic acinar secretion, was also a major trophic hormone for the pancreas.[36] Chronic treatment with this or its analogue caerulein causes pancreatic growth in rats[37-39] and mice.[40 41] Similarly gastrin, which shares the same four C-terminal amino acids as cholecystokinin, when administered as its analogue pentagastrin promotes pancreatic growth.[37 40 42] The physiological role of cholecystokinin in maintaining pancreatic size in health and disease is important.[43] It has recently been shown that promotion of endogenous cholecystokinin is the major agent responsible for pancreatic growth after the addition of soya bean trypsin inhibitor[43-45] and cholestyramine[46] to the diet and also after chronic pancreaticobiliary diversion[46] and jejunal bypass.[47] Plasma concentrations of cholecystokinin are raised in pancreatic atrophy,[48] and caerulein will induce pancreatic hyperplasia in rat models of chronic pancreatic insufficiency[49] and after DL-ethionine diet induced pancreatic degeneration.[50]

Other peptide hormones that promote pancreatic growth include secretin,[37-39] neurotensin,[51 52] bombesin,[53 54] and epidermal growth factor.[55] On the other hand, chronic treatment with somatostatin results in a decrease in resting DNA synthesis of the normal exocrine pancreas within 24 hours of the onset of treatment, with appreciable reduction of total pancreatic DNA content after five days.[56] Caerulein stimulated growth of the normal pancreas is also inhibited by somatostatin.[56]

Glucocorticoids promote pancreatic hyperplasia during suckling and acinar cell hypertrophy at all ages,[57] and this hypertrophic response is both promoted by and promotes the hypertrophic response of acinar cells to the cholecystokinin analogue caerulein.[58] Prostaglandins of the E and F series stimulate pancreatic growth and this response is independent of their effect on serum gastrin release.[59]

Until recently the major problem in understanding normal pancreatic growth has been that all available models have been in whole animal preparations. The only tissue culture in vitro models used cell lines derived from spontaneous or carcinogen induced pancreatic carcinomas. In 1986 Logsdon and Williams reported the methodology for establishing cultures of adult mouse pancreatic acinar cells as monolayers on collagen gels.[60] They were able to show that the cholecystokinin analogue caerulein would directly stimulate growth in vitro,[60] and Logsdon has subsequently shown that cholecystokinin-8 and gastrin, by stimulating the cholecystokinin receptor, promote cell division.[61] In contrast, bombesin, substance P, and carbachol, factors which interact with separate receptors but stimulate pancreatic secretion in the same way as cholecystokinin by mobilising intracellular Ca^{2+}, did not have any effect on the growth of pancreatic acinar cells in vitro. Most recently, Logsdon has shown that epidermal growth factor insulin, and insulin-like growth factor 1, which work through separate intracellular tyrosine kinase pathways, will also induce acinar cell division.[62] Pancreatic ductal cells, dissected from acinar deficient pancreas produced by DL-ethionine deficient diets, have been maintained with secretional integrity in vitro[63 64] for periods of up to six months in tissue culture.[65]

Intracellular mechanisms

The function of regulatory peptides and steroids is to transmit information; however, a specific receptor must exist for each to initiate its own specific response. Molecules of similar structure – for example, cholecystokinin and gastrin, epidermal growth factor and transforming growth factor alpha, insulin and insulin-like growth factor – may in turn act upon unique receptors, related receptors, or the same receptor particularly when they may involve two or more separate responses from the same cell. For example, cholecystokinin will both stimulate acinar secretion of enzymes and promote acinar cell division. The receptor proteins for peptide hormones exist on the cell membrane, whereas those for steroid hormones exist either in the cytosol or within the nucleus.[66] In the case of cholecystokinin the initial site of peptide receptor binding in acinar cells is the basolateral membrane domain and at physiological temperatures (30°–37°C) the bound hormone is subsequently internalised to be localised within specific intracellular compartments where it may have direct actions.[67] Similarly insulin, insulin-like growth factor II, epidermal growth factor, and somatostatin are all internalised in the acinar cells after receptor binding, although to varying degrees and extents.[68 69] The main problem in interpretation of these data is that whereas Scatchard analysis of binding of radiolabelled agonist to receptor ligand will give the number and binding affinity of membrane receptors in each sample, this technique produces a static picture which does not clarify the events occurring beyond the membrane once internalisation has occurred. On the other hand, autoradiographic studies of radiolabelled agonist bound to receptors either on microscopy or after gel electrophoresis of lysed cells will give information about receptor size and site but not about the kinetics of the initial reaction. Thus most of the data derived so far about number of functional receptors per cell at any given moment are probably inaccurate.

Cell receptor populations are not static, constantly being increased ('upregulation') or decreased ('downregulation') by alterations in the rate of receptor synthesis and degradation in response to stimulus drive and target cell response.[66] Downregulation of acinar receptors has been shown in vitro for insulin and acetylcholine.[70 71] Other factors that affect the binding characteristics of specific receptors include occupancy of totally separate hormone-receptor species. For instance, somatostatin enhances binding of oestradiol to its cytosolic protein in the rat pancreas[72] and treatment with cholecystokinin decreases the binding of epidermal growth factor, insulin-like growth factor II, and somatostatin to their respective receptors.[68 69 73]

Chronic treatment with cholecystokinin in vivo, sufficient to cause pancreatic hypertrophy and hyperplasia, does not increase the number of cholecystokinin receptors per cell but the total number of cholecystokinin receptors per pancreas increases.[74] Finally, the number and concentration of cholecystokinin receptors in the pancreas changes with age. During postnatal maturation and weaning in the rat, pancreatic cholecystokinin receptors increase in number.[75] In the guinea pig they reach a peak by 1 year of age and then fall threefold by 3 years of age (median age of survival and equivalent to a 70 year old man).[76]

In general those hormones which stimulate the release of pancreatic enzymes are mediated intracellularly by Ca^{2+} and diacylglycerol while those hormones which regulate ductal secretion are mediated via cyclic AMP,[77] although this division is not absolute.[66] A third class of hormone regulators which are primarily concerned with metabolism and induction of protein synthesis activate tyrosine kinase.[78] Unlike the stimulatory peptides, somatostatin regulates growth by stimulation of tyrosine phosphatase resulting in activated tyrosine phosphatase which in turn activates serine kinase and subsequently inhibits cell division and growth.[79]

Pancreatic cytosolic receptors have been described for glucocorticoids, oestrogens, and androgens.[66 72 80–86] Although oestrogen binding proteins exist in pancreatic cytosol with high binding affinity[80 82] they show different reaction kinetics and are of different molecular size to the high binding affinity oestrogen receptors found in uterine cytosol.[83 86] A separate atypical low affinity oestrogen binding site requiring a peptide cofactor has also been described in the pancreas.[87] Pancreatic oestradiol binding proteins are found in both ductal and acinar cells[83 88] and in these cells act within the region of the endoplasmic reticulum.

Second messengers

As discussed earlier hormone and growth factor signals generally require the stimulation of a second intracellular chemical messenger (transduction) to begin the initiation of a response by the cell. These transduction pathways show autoregulation and directly interact at a number of important modulatory steps.[89] The initial generation of the calcium signal with release of neutral lipids is mediated by the breakdown of phosphatidylinositol and changes in cyclic nucleotide levels involve the activation of adenylate cyclase.

The breakdown of phosphatidylinositol is initiated through the action of a series of phospholipase C's and kinases to produce neutral lipids (which activate protein kinase C), inositol-triphosphate, and arachidonic acid.[90 92] Subsequent studies have shown the action of some transforming oncogenes on phosphatidylinositol turnover,[93] the effect of phorbol esters on cell transformation,[94] and the influence of C-kinase on the epidermal growth factor receptor[95] and transferin receptor.[96] The phosphatidylinositol pathway promotes intracellular Ca^{2+} through inositol 1,4,5-triphosphate and this soluble product is seen immediately after a hormone-receptor interaction which is subsequently mediated via Ca^{2+}.[90 91] Raised intracellular Ca^{2+} activates further enzyme pathways by binding to calmodulin, a calcium binding protein.[89] A rise in the intracellular concentrations of calmodulin have been found in cells undergoing malignant transformation.[97 98] Lastly phosphatidylinositol breakdown generates inositol phosphate

mediators from arachidonate, probably through the action of phospholipase A2 on phosphatidyl choline or phosphatidyl ethanolamine.[94]

The adenylate cyclase system responds to ligand receptor interaction with the generation of cyclic nucleotides, either promoting or inhibiting cyclase activity.[90] The G proteins of the cyclase system influence the affinities of receptors for ligand and so may play a part in receptor sensitisation and desensitisation.[99] Adenylate cyclase has three primary components: G_s, a stimulating subunit; G_i, an inhibiting subunit; and a third, catalytic, subunit.[100]

Nuclear events

Little is known of what occurs after intracellular second messenger promotion during events which stimulate pancreatic growth. More is known, however, about the regulation of pancreatic enzyme production during the same dietary changes described earlier which promote pancreatic growth.[101 102] It seems reasonable therefore to review these pathways since some inference may be drawn from stimulatory events which promote both pancreatic secretion and growth. In the pancreas the synthesis of functional groups of enzymes is regulated by specific hormones, whether these hormones are administered exogenously or produced endogenously.[103]

Production of proteins by the cell, whether for use within the cell or for export, begins with transcription of the DNA code of a particular gene to messenger RNA (mRNA) by RNA polymerase II. Within the nucleus this primary mRNA transcript is processed by either the removal of intron sequences by splicing mechanisms or the addition of a poly (A) tail to the 3′ terminus.[103] The processed mRNA is now transported out to the cytoplasm where, after the functional binding of the 40S and 60S ribosome subunits, the coding sequence of the mRNA is translated into protein.[104] The protein product is then either used or packaged for export. Regulation of gene expression as protein product can therefore occur: (a) at the original transcription of DNA; (b) post-transcriptional processing; (c) rate of mRNA transport; (d) mRNA translation; (e) mRNA breakdown; (f) post-translational processing and cleavage of the protein product; (g) storage of protein product; (h) release or use of protein product. The events which regulate gene expression involve the interaction of regulatory proteins with specific nucleotide sequences on DNA.[105] RNA polymerase II initiates transcription after binding to the promoter (TATA) sequence of the gene which usually lies 30 nucleotides upstream from the DNA nucleotide at which transcription begins.[103] The RNA polymerase then moves downstream from the promoter site, unwinding the DNA and polymerising a single strand of mRNA, regulated further by enhanced gene sequences which lie further upstream in the 5′ flanking region of genes.[103] Enhanced elements either regulate gene expression in a tissue specific fashion or in response to individual hormones.[106] Our knowledge of hormone regulation is, however, largely confined to steroid hormones which have receptor proteins that bind directly to DNA[107] and so regulate gene expression at the level of transcription. Little is understood of the mechanisms of gene expression regulation by peptide hormones and growth factors. Scheele and Kern with their groups have carefully analysed this response by the exocrine pancreas to cholecystokinin and secretin.[108] They have shown that pronounced changes in individual enzyme biosynthesis were not accompanied by changes in mRNA levels, at least during the first six hours of stimulation, suggesting that these initial changes in gene expression are due to alterations in post-translational processing. This time related difference in the mechanism of gene expression is probably because exocrine pancreatic product mRNAs have relatively long half lives of up to six hours and therefore sudden increases in demand for protein synthesis can be met both at and beyond the translational level,[103] which is the rate limiting step in protein synthesis. Changes at this level may involve nucleotide signals in the 5′-non-translated region of mRNAs and the interaction of these signals or sequences with either regulatory proteins or regulatory RNA molecules.[103]

In general, growth factors such as insulin, insulin-like growth factor-1, epidermal growth factor, and platelet derived growth factor all stimulate phosphorylation of the ribosomal protein S6, but the subsequent effect on promoting or inhibiting cell growth and mitosis is cell type specific.[109–113] In 3T3 fibroblasts, treatment with platelet derived growth factor stimulates expression of the *c-myc* and *c-fos* oncogenes,[110] and similar changes in expression of these oncogenes are seen in many cancer cells.

Polyamines

Polyamines (putrescine, spermidine, and spermine) are ubiquitous highly charged cations that are required for growth and differentiation in all eukaryotic cells[114 115] and are particularly important in the regulation of DNA, RNA, and protein synthesis.[116] Membrane stability and cyclic AMP independent protein kinases are apparently also influenced by polyamines.[116] The mechanisms by which polyamines exert their effects are poorly understood, though their polybasic nature has been proposed as being an important factor.[117] High levels of polyamines are found in rapidly dividing cells and a depletion of intracellular polyamines causes a slowing and eventual stopping of cell growth.[118]

The biosynthesis of polyamines in mammalian systems begins with the decarboxylation of ornithine by the enzyme ornithine decarboxylase to form the diamine putrescine. This is the first, and probable rate limiting step, in the polyamine biosynthetic pathway, and ornithine decarboxylase activity is greatly raised during cell division.[119] Subsequently putrescine is converted by spermidine synthase to spermidine, which in turn is converted to spermine by spermine synthase.[120] These last two steps are potentially reversible by polyamine oxidase.[120] During caerulein stimulated pancreatic growth there are increases in content and concentration of all three polyamines which occur within 12–96 hours after the onset of caerulein administration.[121 122] Total

spermidine and spermine content also correlates with the rates of increase in both pancreatic weight and DNA content.[121] Because of their possible antimitogenic potential, many pharmacological inhibitors of polyamine biosynthesis have been developed,[116] most importantly against ornithine decarboxylase because of its key regulatory role.[123] One of these is alpha-difluoromethyl-ornithine (DFMO). DFMO treatment suppresses the increase in pancreatic weight induced by caerulein[124] and this effect can be reversed by administration of putrescine, the biosynthetic product of ornithine decarboxylase.[125] Thus the stimulation of pancreatic DNA synthesis by caerulein seems to be a polyamine dependent process.

The potent phosphodiesterase inhibitor 3-isobutylmethylxanthine causes increases in ornithine carboxylase, putrescine, and *N'*-acetytransferase levels in the rat pancreas and liver. DFMO decreases the accumulation of putrescine in the liver but not the pancreas.[126] These findings suggest that the accumulation of putrescine in the pancreas occurs primarily by the action of the polyamine interconversion enzymes, *N'*-acetyltransferase and polyamine oxidase.

Having reviewed the current opinions on the regulation of normal pancreatic growth we now consider the present views on the abnormalities and regulation of growth in pancreatic cancer.

Aetiological factors of pancreatic cancer in humans

Exocrine pancreatic cancer is a disease of Western society with a tenfold difference in prevalence between countries where the disease is common (UK, USA) and countries where it is less frequent (India, Nigeria).[1 127] There are also racial predilections with blacks in the Bay Area of California having 1·5 times the risk of developing the disease as whites in the same area; however, diet and environment also play a part as American blacks have a greater chance of developing the disease than African blacks.[127] Within the United States the incidence of pancreatic cancer in blacks is five times greater than that of Japanese Americans or Hispanics from Puerto Rico.[127]

Other established risk factors include age and sex. All studies show the disease to be commoner in men than in women, with a ratio ranging from 1·5:1 to 2:1 depending on the community.[128] This may be hormonal, but men are also exposed on the whole to other risk factors for the disease. There is an established link between pancreatic cancer and cigarette smoking; it seems likely that nitrosamines may have a causal role.[127] Whether these chemicals reach the pancreas directly or, after hepatic metabolism, are excreted in the bile and so affect the pancreas is unknown. Smoking also raises serum lipids which may predispose to pancreatic cancer, since high fat diets are also related to the increased incidence of the disease.[1] Other factors associated with an increased incidence of pancreatic cancer include working in chemical industries (particularly beta-naphthylamine and benzidine), petroleum plants, coke and coal gas plants, dry cleaning industries, and plants handling radioactive materials for nuclear fuels and warheads.[127] An unsubstantiated risk factor is urban living. Earlier studies between 1935 and 1974 suggested this association but intensive county-by-county studies performed in Connecticut and Iowa in 1974 did not bear this out.[127]

Coffee and caffeine have been linked to pancreatic cancer but this link is tenuous since such drinking habits are often linked to other risk factors like cigarette smoking. Also, the incidence of coffee drinking tends to increase during the early stages of the disease when patients become anxious about insidious symptoms or develop glucose intolerance.[1] Similarly, excessive alcohol consumption does not predispose to pancreatic cancer. Gall stone disease and chronic pancreatitis have been linked to the development of pancreatic cancer but close analysis of the data, combined with better diagnostic criteria, have challenged this connection.[127 128] A causal relation with diabetes (which at one time was thought to increase the chance of developing pancreatic cancer threefold) has been confused by the fact that up to 15% of patients with pancreatic cancer will have developed diabetes because of their cancer in the immediate period before presentation. When this factor is taken into account, there is no difference in the prevalence of pancreatic cancer between diabetics and non-diabetics.[129]

Aetiological factors of pancreatic cancer in animal models

The adaptive changes of hypertrophy and hyperplasia in the pancreas are diffuse and reversible; on the other hand, neoplastic growth is clonal and involves multiple steps through preneoplastic changes to produce a tumour.[130] Completion of the sequence of changes to irreversible malignant growth usually requires a major portion of the lifespan of the host, and the rate of progression of preneoplastic lesions to cancer may be regulated by the same factors that control adaptive growth. The major difference between neoplastic growth and adaptive growth is that the former is the result of altered division of single cells (clonal growth) and the latter represents the regulated growth of the whole organ. Certainly in the two rodent models of pancreatic cancer (azaserine induced acinar cancer in the rat and *N*-nitrosobis(2-oxopropyl)amine induced ductal cancer in the hamster) the sequence of progression is: initiated cell→focus→nodule→neoplasm (carcinoma).[130] The sensitivity of cells to carcinogenesis is promoted if the cells are already undergoing mitosis – rats are highly sensitive to the induction of preneoplastic acinar lesions when carcinogens are given during the first three weeks of life[131]; during pancreatic regeneration[132]; after pancreaticobiliary diversion[133 134]; and after administration of exogenous or a rise in endogenous cholecystokinin.[130 135-137]

As in humans, dietary lipids promote the growth of carcinogen induced pancreatic cancer in rodents. Diets that include 4–8% unsaturated linoleic fatty acid enhance the development of azaserine induced acinar cancer in Lewis rats,[138] and lithocholic acid when combined with

cholecystectomy produced greater numbers of *N*-nitrosobis(2-hydroxypropyl)amine ductal tumours in hamsters.[139]

Endocrine regulation of pancreatic cancer

The idea that cancers which arise from hormone target organs possibly maintain their hormone responsiveness has evolved into the classification of cancers as hormone dependent or independent and has led to the development of treatment regimens aimed at arresting tumour growth by either hormone deprivation or administration of antagonistic agents of these hormones. The latter would include treatment using other hormones whose action would oppose those hormones which promote tumour growth. The concept of growth factor and hormone regulation of cancer has been extensively and recently reviewed by Goustin and colleagues[140] and Townsend and coworkers have specifically reviewed the role of growth factors in intestinal neoplasms.[141]

Direct effects of hormones on pancreatic carcinogenesis

Importantly, a chronic rise in plasma cholecystokinin by longterm feeding with raw soya flour (for more than four months) will cause hyperplastic foci of pancreatic acinar cells.[142 143] If this diet is discontinued after 24 weeks then pancreatic morphology will subsequently revert to normal; however, if the diet is continued for up to 36 weeks some of these hyperplastic foci will progress to pancreatic cancer, even without the addition of carcinogens.[143] The link between cholecystokinin and carcinogenesis, however, is not straightforward since Pour and colleagues have shown in the hamster model of pancreatic ductal carcinogenesis that exogenous cholecystokinin, given simultaneously with or shortly before the carcinogen *N*-nitrosobis(2-oxopropyl)amine, inhibited cancer induction.[144-146] On the other hand, Satake and colleagues found that weekly administration of caerulein enhanced the carcinogenic effect of *N*-nitrosobis(hydroxypropyl)amine.[147] Similarly, Howatson and Carter found that both cholecystokinin[148] and secretin[149] enhanced *N*-nitrosobis(2-oxopropyl)amine pancreatic carcinogenesis in hamsters. It may be that the studies which failed to show any effect used insufficient dosages of caerulein to achieve the hyperplastic response necessary for carcinogenesis, but the inhibitory studies may also relate to the phenomenon that caerulein also inhibits the growth of a human cholangiocarcinoma, known to possess cholecystokinin receptors, when growing in nude mice.[150] Epidermal growth factor promotes *N*-nitrosobis(2-oxopropyl)-amine pancreatic carcinogenesis in hamsters[151] and both the somatostatin analogue RC-160 and luteinising hormone releasing hormone reduce *N*-nitrosobis(2-oxopropyl)amine pancreatic carcinogenesis and prolong survival in hamsters.[152-154]

Hormone regulation of established tumours

CHOLECYSTOKININ AND SECRETIN

There are good data to show that cholecystokinin will promote the growth of established pancreatic cancers in the same way that cholecystokinin stimulates normal pancreatic growth. Townsend and coworkers reported that the combination of caerulein and secretin (but neither agent alone) produced significant stimulation of growth in a transplantable hamster pancreatic ductal cancer (H2T).[155] Receptors for both hormones have been found in a human pancreatic cancer cell line.[156] Asperlicin, a competitive non-peptide cholecystokinin antagonist, significantly inhibits the growth of a human pancreatic adenocarcinoma (SKI), established in nude mice in Dr Courtney Townsend's laboratory in Galveston, that possesses cholecystokinin receptors,[157] probably by inhibiting the effect of endogenous cholecystokinin.[157] A similar effect is seen with the cholecystokinin antagonist CR-1409 on exogenous cholecystokinin promoted tumour growth in azaserine induced rat pancreatic cancer.[158] Upp and colleagues reported that the presence of cholecystokinin receptors on a human pancreatic cancer will predict the response of the cancer to caerulein treatment.[159] Caerulein produced a pronounced stimulation of growth in the tumour possessing cholecystokinin receptors and this effect was inhibited by proglumide, the gastrin-cholecystokinin antagonist. Growth of the tumour without cholecystokinin receptors was not affected by treatment. Furthermore, caerulein treatment enhanced the presence of high affinity binding receptors for cholecystokinin in the SKI tumour.

BOMBESIN

Bombesin, a member of the gastrin releasing peptide family of hormones, normally promotes pancreatic growth[160] and indeed will promote the growth of azaserine induced rat acinar tumours.[158] Chronic treatment with bombesin, however, inhibits the growth of human pancreatic cancer in nude mice while stimulating the growth of the normal nude mouse pancreas.[161] This demonstration of simultaneous trophic and inhibitory responses by the same hormone on normal and malignant tissue suggests that the action of bombesin, directly or indirectly through the hormones that it stimulates, may be site specific, interacting differently in normal and neoplastic tissues. Possible explanations for this phenomenon may be that different dosages of the same hormone may act differently: multiple forms of hormone receptor may exist to serve different functions (note the effects on growth and secretion discussed earlier); hormones may act directly on target cells through hormone-receptor regulation (homologous or heterologous); or hormones may act indirectly by releasing other hormones or growth factors (autocrine, paracrine of endocrine) to produce their response from the target cell.

VASOSACTIVE INTESTINAL PEPTIDE

Vasoactive intestinal peptide receptors exist on both normal[162] and neoplastic[163] pancreatic tissue. Binding of the peptide to these receptors promotes intracellular cyclic AMP accumulation,[164] which may be a regulatory factor in cell division

and growth.[165-167] Poston and colleagues have shown that chronic treatment with vasoactive intestinal peptide inhibits the growth of hamster pancreatic cancer in vivo but not human pancreatic cancer.[168] This effect may be related to the presence of two vasoactive intestinal peptide receptor types (one with molecular weight 66 kDa, and the other of 90 kDa) on the hamster pancreatic cancer cells whereas only one type of peptide receptor (MW 66 kDa) was detectable on the human cancer cell lines.[163]

SOMATOSTATIN

Somatostatin inhibits the growth of several malignant tumours and tumour cell lines[169-173] and its effect on pancreatic carcinogenesis has been described.[152-154] The oncological application of somatostatin and its analogues have recently been extensively reviewed by Schally and colleagues.[174-176]

In 1984 Redding and Schally described the inhibition of both rat and hamster pancreatic cancer by administration of somatostatin.[177] Subsequently Upp and colleagues have reported that the somatostatin analogue SMS 201-995 inhibits the growth of human pancreatic cancer in nude mice.[178] Recent reports suggest that this response may be potentiated by the oestrogen antagonist tamoxifen.[179] In vitro, somatostatin reverses the growth potentiating effect of epidermal growth factor on MIA PaCa-2 human pancreatic cancer cells[79] and H2T hamster ductal pancreatic cancer cells (unpublished data from our laboratory), which may be through promotion of tyrosine phosphatase activity acting on the epidermal growth factor receptor.[79]

Not only does somatostatin treatment prolong tumour doubling time[178] but it may result in programmed cell death (apoptosis) in carcinogen induced pancreatic cancer in hamsters, in some cases completely preventing the development of carcinogen induced tumours.[180] Although evidence exists from in vitro studies for a directly mediated response,[181 182] somatostatin may also affect tumour growth in vivo by inhibiting other hormones such as cholecystokinin which may promote tumour growth.[183] We have recently shown somatostatin analogue inhibition of epidermal growth factor and transforming growth factor alpha stimulated growth of pancreatic cancer in a hamster model (unpublished data from our laboratory). Somatostatin receptors have been shown on a well differentiated cell line derived from rat carcinogen induced acinar cancer AR42J.[184] Under the auspices of the Cancer Research Campaign, we have now started clinical trials on the use of long acting somatostatin analogues for treatment of pancreatic cancer but care will be necessary because of the potential side effects on other gastrointestinal pancreatic hormones, and in particular on the management of serum glucose.[185]

Epidermal growth factor and transforming growth factors

As described earlier, epidermal growth factor promotes pancreatic carcinogenesis in hamsters[151 186] and promotes the growth of human pancreatic cancer cells in vitro.[79] Retrovirus insertion of epidermal growth factor receptor gene into 3T3 fibroblasts is associated with malignant transformation, the degree of which is proportional to the extent of epidermal growth factor receptor expression.[187] Carcinoma cells which overexpress the epidermal growth factor receptor produce a 270 kDa epidermal growth factor receptor gene transcription factor[188] and that form of this factor can result in tenfold gene expressions of epidermal growth factor receptor gene.[189] DNAase 1 footprinting studies have identified four binding sites for these transcription factors on the epidermal growth factor receptor gene promoter.[190]

Normally, stimulation by epidermal growth factor downregulates its receptor expression, so regulating the number of available epidermal growth factor receptors[191-193] with phosphorylation of membrane proteins.[194] The pancreas is one of the richest sources in the body of mRNA for prepro-epidermal growth factor,[195] so raising the question of autocrine growth stimulation of pancreatic cancer by epidermal growth factor production.[196] The epidermal growth factor receptor is the cellular homologue of the avian erythroblastosis virus *erb-B* proto-oncogene.[197 198] The gene for pancreatic neuropeptide Y and *erb-B* overlap on human chromosome 7[199] and Korc *et al* have recently shown that human pancreatic cancer cells overexpress the gene for epidermal growth factor receptor associated with concomitant changes on chromosome 7.[200] These increases in expression of epidermal growth factor receptor may be as great as 100-fold.[201 202]

Normal fibroblasts must attach to a surface to grow in vitro. If exposed to substances which reversibly transform them, however, these same fibroblasts can grow in suspension; such factors have been termed transforming growth factors (TGF).[203 204] Two groups of these growth factors have been identified. TGF α, 5·6 kDa polypeptide structurally related to epidermal growth factor with which it shows a 35% homology by possession of six cysteine residues in the same relative positions, and can thus bind to and stimulate the epidermal growth factor receptor.[205] The TGF βs are a separate distinct polypeptide family of about 25 kDa in size and have no sequence homology with TGF α.[203 206 207] TGF βs do not bind to the epidermal growth factor receptor[208] but stimulate a separate unique receptor to induce *c-sis* oncogene mRNA expression which in turn induces a mitogenic response through production of platelet derived growth factor.[209] But unlike their effect on mesenchymal cells, TGF βs have a different effect on epithelial cells, being the most potent growth inhibitory peptide known for a number of epithelial cells.[210 211] Interestingly, transfection of mouse fibroblast cells with a *c-myc* oncogene results in acquisition of responsiveness to TGF β.[212] Both TGF α and β are produced by breast[213 214] and colon cancers.[215]

Several cultured human pancreatic cancer cell lines produce TGF α in addition to epidermal growth factor[201 205] and TGF α is 10–100 times more potent than epidermal growth factor in stimulating anchorage independent growth of

these same cell lines.[205] TGF α binds to pancreatic epidermal growth factor receptors where it is degraded after receptor stimulation.[205] The binding of TGF α to the epidermal growth factor receptor does not however downregulate the expression of the epidermal growth factor receptor as occurs normally with epidermal growth factor stimulation[191-193 205] and so may result in an uncontrolled autocrine growth effect. This effect can be reversed by culturing the cells on soft agar containing anti-TGF α monoclonal antibody.[205] On the other hand, some pancreatic cancer cells have been shown to produce TGF β, which in turn inhibits their own growth.[205] These findings of Korc's group suggest that TGF α participates in the regulation of pancreatic cancer cell proliferation and point to the possible existence of a TGF α epidermal growth factor receptor autocrine cycle whose function may be modulated by TGF β.[216] We have just shown that exogenous administration of TGF α stimulates the growth of hamster pancreatic cancer in vivo (unpublished data from our laboratory).

STEROIDS AND LUTEINISING HORMONE-RELEASING HORMONE

Recent interest has focused on the use of steroid hormones and their antagonists in the treatment of pancreatic cancer.[217 218] Certainly, oestrogen receptors can be shown on normal[219 220] and neoplastic[220-225] pancreatic tissue in both human and carcinogen induced rat acinar tumours. Androgen receptors are found on both normal pancreas[226] and pancreatic cancers[227] and both glucocorticoids[228 229] and sex steroid hormones have a regulatory role in normal exocrine pancreatic secretion and growth.[230] When given to spayed female mice relatively high amounts of the active metabolite of tamoxifen, 4-hydroxytamoxifen, have been found in the normal pancreas as long as 24 hours after administration,[231] and these findings have formed the basis of early clinical trials in patients with pancreatic cancer which have claimed some limited success for antioestrogen[232] and luteinising hormone-releasing hormone antagonists to suppress endogenous oestrogen.[233 234]

In the laboratory it is much more difficult to induce pancreatic cancers using azaserine in female rats than it is in male rats,[235] but this can be overcome with prior oophorectomy and tamoxifen treatment.[235] Similarly, oestrogen treatment and castration inhibit the early stages of acinar pancreatic carcinogenesis after azaserine treatment in male rats.[236] In human pancreatic cancer cells there is good evidence that 4-hydroxytamoxifen produces its growth inhibitory effect through specific antioestrogen binding sites,[237 238] although these effects bear little relation to oestrogen receptor content.[238]

Plasma concentrations of circulating androgens are decreased in patients, particularly men, with pancreatic cancer,[239-243] and this has led some to claim that low concentrations of testosterone may be an endocrine marker for the disease.[241 243] Certainly studies on rat pancreatic cancer[244] and human pancreatic cancer in nude mice[245 246] and tissue culture[238 246] show a trophic effect of androgens on the growth of the tumour cells. This may be a factor in the predilection of the disease for males in general.[1] In male hamsters, however, castration promotes the growth of transplantable ductal cancers but this may be mediated through the tumour promoting effect of castration by increasing the release of endogenous gastrin.[247] Testosterone may act either directly on the cancer cells or be converted by aromatase to oestrogen or by 5-α-reductase to the more potent 5-α-dihydrotestosterone, after internalisation into the pancreatic cancer cells.[248]

Glucocorticoids stimulate the growth of human pancreatic cancer cells and rat AR42J acinar cancer cells in vitro[238] and also promote the synthesis of amylase mRNA levels, secretory organelles, and enzyme secretion in AR42J cells in tissue culture.[249] Again, there is variance in transplantable hamster ductal pancreatic cancer where dexamethasone treatment inhibits tumour growth (unpublished results).[250]

Intracellular events

Little is known about oncogene activity and the role of oncogenes in human pancreatic cancer. Cooper and colleagues have characterised expression of activated *ras*^{K} gene in human pancreatic cancer cell lines[250] and activation of this gene appears to be due to an amino acid change on position 12 of the *ras*$^{K-2}$ sequence.[251]

Palmiter and Brinster have created a transgenic model of acinar pancreatic cancer in mice by fusing the SV-40 tumour virus to the elastase 1 gene, which is only expressed in the pancreas.[252] These mice develop pancreatic cancer by three months of age and will prove a powerful tool in the dissection of molecular events leading to the induction of pancreatic cancer.

Ornithine decarboxylase activity is greatly raised in hamster pancreatic cancer cell lines[253] and inhibition of this with DFMO inhibits the growth of both human and hamster pancreatic cancers.[254 255] This effect is reversed by the administration of exogenous putrescine[254] and potentiated by the addition of cyclosporine both in vitro[256 257] and in vivo.[258]

Tumour cells characteristically exhibit an increased rate of glycolysis,[259] the so called 'Warburg effect,' but little is known of the regulation of this mechanism in pancreatic cancer cells. Schek and colleagues have shown by using complementary DNA (cDNA) probes that there is an overexpression of mRNA for glyceraldehyde-3-phosphate dehydrogenase, enolase, and glucose transporter protein which may play a part in the Warburg effect.[260]

The ability of malignant tumours to invade normal tissue is dependent on the production of proteases such as collagenase, plasminogen activators, and lysosomal proteases. The human pancreatic carcinoma cell line HPC-YT secretes excessive amounts of acid proteases which may play a part in this process.[261]

Finally, much clinical interest has focused recently on the production of antigen markers by pancreatic cancer. In 1979 Kaprowski and colleagues reported that raised levels of CA19–9, a carbohydrate antigenic determinant were present in sera of patients with gastrointestinal cancer[262] and subsequent workers have shown

that it is raised in the serum of as many as 80% of patients with pancreatic cancer.[263 264] Pancreatic cancer can also produce carcinoembryonic antigen,[265] tumour associated glycoprotein (TAG 72), and DU-PAN-2.[266]

Conclusion

Much is known about the physiological, pharmacological, and surgical events which will promote pancreatic growth and carcinogenesis. We are beginning to understand the intracellular events which regulate normal pancreatic hypertrophy and hyperplasia. Although we now know that pancreatic cancers will respond in many similar ways to these events, there are vast gaps in our knowledge of the intracellular events which occur after pancreatic carcinogenesis. Certainly it is well established that there is a wide variability of biology and response to treatment within pancreatic exocrine cancers as has been carefully shown by Klapdor's group.[267 268] The greatest challenge to pancreatic oncology during the next decade will be in dissecting out these intracellular mechanisms and differences in biology and establishing where the disorders of regulation exist.

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