Article is available online at http://www.webio.hu/por/2003/9/4/0252

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Pancreatic Cancer – a Continuing Challenge in Oncology

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Pancreatic cancer is still one of the major health problems because of its rising incidence and the modest therapeutic results. The paper surveys the statistical data, the risk factors, the preneoplastic ductal lesions, the hormonal sensitivity, the possible transdifferentiation in the endocrine and exocrine parts and the possibilities for chemoprevention. Hungary is peculiar among the European countries because during the last 50 years the incidence of pancreatic cancer has displayed a 15-fold increase. Apart from smoking, additional risk factors seem to be important, and recently a puzzling association between Helicobacter pylori seropositivity and pancreatic cancer was found. First-degree relatives of patients with pancreatic cancer are also at increased risk of this tumor. The term pancreatic intraepithelial neoplasia (PanIN) seems yet to be established, but the dynamics of these lesions needs to be further elucidated. Several lines of firmly established data indicate the hormonal sensitivity of this tumor, but still an unexplained discrepancy exists between the experimental and the clinical results. In addition to the somatostatin analogs, anti-gastrin vaccine is being tested. The mixed exocrine-endocrine tumors might suggest a real possibility of transdifferentiation between different compartments of the pancreas. Finally, the paper outlines the available data about the possibility of chemoprevention, including the role of cyclooxygenase inhibitors. (Pathology Oncology Research Vol 9, No 4, 252–263)

Keywords: pancreatic cancer, risk factors, PanIN, hormones, chemoprevention, review

Introduction

Despite notable – and sometimes spectacular – achievements in many fields of oncology during the last decades, pancreatic cancer (PC) still remains one of the major public health problems. First, it is among the ten most frequent malignancies worldwide including Hungary. Second, its incidence has steadily been increased in most countries and third, this type of tumor has a grim prognosis because the survival rates are disappointing. Although experimental data have been accumulated ranging from epidemiology to the molecular level, it is clear that we still do not understand the pancreatic carcinoma. In this review we attempt to survey selected topics of the pancreatic cancer: major problems, intriguing facts, controversies and some promising approaches.

Received: Nov 12, 2003; *accepted:* Nov 26, 2003

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This study was financially supported by the National Research Found Grant (OTKA T 34899.)

Statistics

A rising incidence of this tumor has been reported from different countries of the world without a characteristic geographical distribution pattern. Around the turn of the 20th century pancreatic carcinoma cases accounted just for about 1.2% among malignant tumors,[33] but nowadays this figure is around 5%. Large-scale retrospective studies (covering 35-70 years) unequivocally revealed an increased mortality rates in both sexes. This tendency was observed in Australia,[116] Japan,[60] Norway,[32] Switzerland,[74] and USA.[75] For example, in Japan between 1955 and 1993 the age-adjusted mortality has increased 5.1 times for men, 4.3 times for women. Similarly, nearly a 3-fold upward trend was observed in the USA between 1920-1978, but since that time the rates have remained constant. By contrast, in nearly all European countries the pancreatic cancer incidence has continued to rise, although the rates

are highly variable (ranging from 6% vise in Scotland to 279% in Spain.)[37]

A century ago, in Hungary – like in other countries – this type of tumor was a rare disease. In a Budapest-based large autopsy material (38 252 cases) during a 40-year-period (1896-1935) E. Zalka was able to find only *107* pancreatic carcinomas! In 1948 the mortality rate of pancreatic cancer was 1.1 per 100 000 inhabitants, but since that a continuous and steep increase has been observed reaching up 16.4/100 000 (*Figure 1*.) This 15-fold rise is among the highest in Europe, but its explanation is totally obscure, especially in the light that the surrounding countries do not display such a rapid elevation. In Austria, for example, there was only a threefold increase between 1928 and 1972,[94] or in Slovakia the annual change from 1968 to 1977 was about +0.2%.[98]

Is there any progress in the prognosis? Yes, there is, but very modest. Fifty years ago in the United States the 5-year survival was only 1%, 25 years later it was 3% and between 1992 and 1998 statistics showed 4%.[62,142] In other words, the absolute increase of survival between 1950 and 1998 was just 3 % – this figure is by far the lowest among the 20 most frequent malignancies. By comparison, during this period of time the change in 5-year survival proved to be up to 50% for prostatic cancer, 39% for melanoma, 26% for breast cancer, 20% for colorectal cancer, etc.[142] The overall European results are similarly poor: in the period of 1978-1989 the average survival data show also 4% survival benefit with some inter-country differences.[34]

The grim prognosis of pancreatic cancer is mainly (but not exclusively) dictated by the fact that the majority of cases are discovered at an advanced stage. According to US data only 8% of cases are localized,[62] 57% presents with stage IV,[61] and 49% of patients receive no cancer-directed therapy.[61] Patients with unresectable pancreatic cancer live about 6 months, the 5-year survival for resected cases is about 10-19% with a median survival of 12-18 months.[1,43,100] In recent years more patients are operated. A number of authors claim a 5-year survival rate after resections of 30-58%. These figures, however, may be due to statistical bias, the real proportion of survivors must be much lower.[46] It is also important to emphasize that only histologically proven cases must be taken into account, because Finnish authors have demonstrated many cases among the long-term survivors where the diagnosis had been based on macroscopic (operative) findings only.[2]

As was mentioned before, the staging is the most important but not the only factor determining the prognosis of pancreatic cancer. Analyzing around 17 000 pancreatic cancer cases Janes et al. have clearly demonstrated that the survival was uniformly poor (8% for stage I, 3% for stage IV)[61] suggesting that the biological behavior of this type of carcinoma is basically aggressive regardless of the tumor burden.

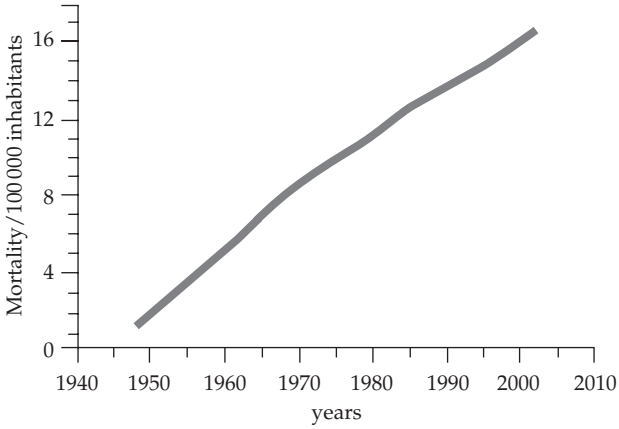

Figure 1. *Mortality trends of pancreatic cancer in Hungary between 1948 and 2002 displaying a 15-fold increase in incidence.*

Risk factors

Although the etiology of pancreatic cancer is still poorly understood, environmental factors, especially smoking have been sharply implicated by epidemiological studies. In most studies cigarette smokers display a 2 to 3-fold increase of relative risk and at least 15 years of abstinence has to elapse until the risk declines again to the level of non-smokers.[10] Among the carcinogenic materials found in the cigarette smoke the tobacco-specific nitrosamines (TSNA) are of outstanding importance. In addition to the lung, esophagus and oral cavity, human pancreas is also exposed to TSNAs.[53] Some of these compounds have also been identified in the pancreatic juice from smokers, together with cotinine, a major metabolite of nicotine.[103] Aromatic amines can also be metabolized by pancreatic microsomal enzymes,[3] and a significantly higher level of aromatic and lipid-peroxidation DNA-adducts were detected in human pancreatic cancer samples compared with controls.[139] Occupational exposure does not seem to be a major contributor,[66] although several studies have revealed positive correlation between the occurrence of this tumor and some chemical compounds. The relative risk was found to be increased among workers with high level of chromium,[141] DDT,[41] halogenated hydrocarbons,[6] plastic manufacturing[25] or vinyl processing.[122] Pesticide exposure has also been implicated: among long-term residents or aerial pesticide applicator pilots the pancreatic cancer mortality was found to be elevated but without a consistent dose-response relationship.[15,18]

Lifestyle factors have also been suspected. During grilling or barbecuing red meat a number of carcinogenic materials (heterocyclic amines, polycyclic aromatic hydrocarbons, etc.) can be formed and this cooking technique should also be regarded as a risk factor.[5] Slight, but not consistent positive associations have also been

observed between *saturated fat/cholesterol* consumption, excess energy derived from fat and pancreatic cancer,[42] although the risk is just weakly associated with obesity.[23] Gallstones or cholecystectomy have no impact on the subsequent development of pancreatic cancer.[48] There has been much debate about the association of *alcohol* drinking and pancreatic cancer. The main problem in these investigations that it is very difficult to separate the confounding effect of smoking, but nowadays its causative role seems to be unlikely. Large population-based epidemiologic studies have not revealed an increased risk among drinkers,[76,86] and similarly, no statistically significant associations were observed for intakes of tea, total or decaffeinated coffee consumption.[86]

The significance of *chronic pancreatitis* is likewise a debatable and unsettled issue. Pros and cons can be found equally in the world literature. It gains increasing popularity as a facultative pre-neoplastic condition, although chronic fibrotizing inflammation is relative frequently seen surrounding the carcinoma. In some prospective studies, pancreatic cancer occurred in 3-4% of patients with chronic pancreatitis and this figure was much higher than in the control cohort groups.[120,138] Several authors claimed that the K-ras mutation (at codon 12) which is nearly universal finding in pancreatic carcinoma was also frequent (25-42%) in chronic pancreatitis cases,[90,105,136] but other studies failed to reinforce these results.[79,93,130] On the other hand, in patients suffering from chronic pancreatitis chromosome-instability could be detected that favors the process of carcinogenesis.[19] The most intriguing study was published by Finnish authors: during a 19-year follow up of a notable number of patients with chronic pancreatitis, thery found a moderate (3.8-fold) increase of carcinoma risk, however, just in the first decade, but in the cases with a chronic pancreatitis history of more than 10 years the relative risk proved to be identical with that of the normal population.[31] If chronic pancreatitis was really a pre-neoplastic condition displaying various genetic abnormalities, the carcinoma incidence should be progressively increased with time.

An interesting but still unexplained association was also found with *Helicobacter pylori* seropositivity. The causal role of this bacterium in the pathogenesis of gastric MALT-lymphoma is firmly established, and we know that it effects on exocrine pancreatic physiology,[83] but the relation of the infection to pancreatic carcinoma is puzzling. The first such study was performed in Vienna: 65% of pancreatic cancer patients tested by ELISA were found to be seropositive compared to controls (47%).[106] Similarly, it was demonstrated that subjects with H. pylori or cytotoxin-associated gene-A positive strains were at elevated risk of pancreatic cancer.[125] Although no microscopical presence of the organism could be detected and it is generally believed that the bacterium is not able to colonize the pancreas, PCR for Helicobacter was positive in 5 of 6 pancreatic carcinomas.[91] Although these data are intriguing and fragmentary this issue deserves further studies because both the H. pylori infection and the pancreatic cancer represent significant medical problems.

An another exciting problem is *familiarity*. As a matter of fact, most pancreatic cancer cases do occur sporadically, but, it has long been recognized that there are families in whom this tumor developed in several members conveying the suggestion of inheritance. After anecdotal case reports some countries (USA, Germany, Sweden, etc.) have established national registries to analyze these cases, the largest such a collection being at Johns Hopkins University (more than 1100 families). Although we are still far from understanding the development of familial carcinomas, some important data have been accumulated. In the United States approximately 5-10% of patients with pancreatic cancer have a family history of the same tumor,[69] but this figure in Sweden is seems to be much lower (1.1%).[54] Positive medical history confers up to 13-fold increased risk of developing pancreatic cancer,[68] but when more than 3 family members suffer from this tumor, the chance of developing PC in an another person rises to more than 50-fold.[58] Interestingly enough, these families are at increased risk of developing not just pancreatic, but also breast, lung, bladder, prostatic carcinomas or malignant melanomas. Among the familial pancreatic cancer group a small fraction can be further separated in whom the disease arises in another inherited cancer syndromes (familial atypical multiple mole-melanoma, Peutz-Jeghers syndrome, hereditary breast-ovarian cancer syndrome, etc.).[69] Peutz-Jeghers syndrome also predisposes to an increased risk of PC.[126] Thus, the familial pancreatic cancer represents a heterogeneous group of disease.

What genetic abnormalities underline familial pancreatic cancer is unknown in up to 80% of the cases. It is clear now that it that transmission appears to be complex, not representing a mendelian disorder and it has not been linked to defects in any single specific gene.[50] Rather, several malfunctioning genes might render the pancreas susceptible to carcinoma formation. The most frequently involved such genes are the p16, BRCA2, hMLH1, PRSS1, STK11 and a "familial pancreatic cancer gene chip" is being developed to speed up research in this field.

Although collection and statistical analysis of pedigree data have suggested that genetic mechanisms might play an important role in these families, the noxious environment aggravating or accelerating the neoplastic process cannot be excluded either. These interrelationships are almost totally obscure, but for example, we do know that smoking enhances the risk of familial pancreatic cancer kindreds, because smokers develop cancer 10 years earlier than the non-smokers.[119] Moreover, several reports

showed significant associations for parental occupations involving harmful chemicals and development of malignancies in their children.[7]

Ductal alterations preceding carcinoma formation (Premalignant alterations of pancreatic cancer)

Development of pancreatic carcinomas – similarly to other malignant tumors – is a multistep process requiring gradual accumulation of increasing number of genetic abnormalities. Various ductal changes long have been known in the vicinity of the main bulk of the tumor and they have been classified as different types of *hyperplasias* (simple, papillary, atypical).[16] However, there was a disagreement about their nature: some authors claimed that they could be just secondary phenomenona due to obstruction resulting from the tumor itself,[20] but others regarded them as real pre-neoplastic conditions. There was also a chaos around the terminology: pancreatic experts used more than seventy (!) different diagnoses describing these lesions. Emerging molecular pathological studies have led to the better understanding the whole process. *Day et al*. have reported that the immunohistochemical expression of HER-2 (c-erbB-2) is negative in normal pancreatic ducts, but in the flat and papillary lesions, in atypical hyperplasia or in the cases of in situ carcinomas the receptor is present in 82-100%.[22] The value of K-ras or p53 mutations in this respect proved to be inconclusive because these changes were frequently found also in chronic pancreatitis. Ki-67, however, was a reliable marker: in normal ducts positive reaction was found in 0.41%, in simple or papillary hyperplasia in 0.69 - 2.3%, in atypical hyperplasia in 22%, while invasive carcinoma exhibited a 37% of nuclear positivity.[70] Similar progression was observed in the p21 expression: from 9% of the normal ducts through the hyperplastic/preneoplastic lesions to the 85% of the pancreatic carcinoma.[8] The same group has also reported an increased proportion of cyclin D1 positivity and the lack of DPC4/Smad4 protein expression in these ductal changes.[8] These and similar results has led to the concept of the pancreatic intraepithelial neoplasia.

In 1994 when *Klimstra* and *Longnecker* first proposed that the different "hyperplasia" be replaced by the term *pancreatic intraepithelial neoplasia* (PanIN),[71] but the detailed pathological nomenclature has been elaborated years later.[57] In this classification PanIN-1A, PanIN-1B, PanIN-2 and PanIN-3 definitions are used. In the earliest stage of the process (PanIN-1A) the cuboidal cells become tall, columnar and they are characterized by the presence of large amount of intracytoplasmic (especially alcian-blue-positive) mucin that is regularly absent in the normal ducts. Goblet cells may or may not be present. (*Figure 2a*). In PanIN-1B the cellular alterations are the same but there are many papillary infoldings (*Figure 2b*). Rarely, in the basal layer some pseudostratification can be observed, but the nuclei are regular, smooth-contoured, and signs of atypia are missing. In PanIN-2, however, (which may be flat or papillary) the cellular abnormalities are already evident: slight loss of polarity, crowded nuclei, increased basophilia. Mitotic figures may appear, but always deeply and they are not atypical. A cribriform pattern is never seen is this category (*Figure 2c*). The most severe changes are observed in PanIN-3: this lesion is typically papillary or micropapillary in appearance, small groups of cells exhibit a "budding" into the lumen or real cribriform pattern is seen. At the luminal surface necrotic areas may be present. All the cytological signs of atypia are clearly evident, the nuclei are pronounced, not infrequently abnormal mitoses are shown, but the basal membrane is continuous and intact. (*Figure 2d*) In the older terminology this alteration was designated as a severe dysplasia, in situ carcinoma or intraductal carcinoma.

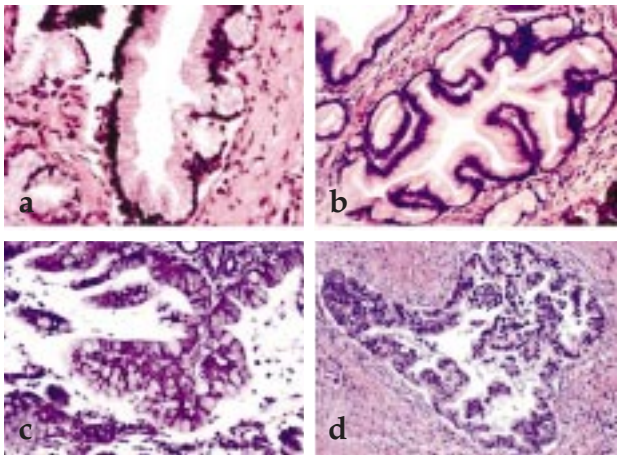

Figure 2. *Histological spectrum of the pancreatic intraepithelial neoplasia (PanIN).* ***(a)*** *PanIN-1A: flat mucinous hyperplasia in the small ducts (HE, x200);* ***(b)*** *= PanIN-1B: papillary mucinous hyperplasia without any nuclear changes (HE, x100);* ***(c)*** *= PanIN-2: in the papillary proliferation the nuclei are slightly enlarged, hyperchromatic, and pseudostratification is seen (HE, x150);* ***(d)*** *= PanIN-3: many papillary infoldings are seen, the cells are severely atypical, the nuclear crowding and polymorphism is a characteristic finding (HE, x40).*

It should be emphasized that this nomenclature refers to the alterations occurring in the small/intermediate ducts because the papillary-mucinous tumor that involves the main pancreatic ducts represents a separate entity.

A logical, but still unanswered question is the natural history: what proportion of PanIN lesions becomes frankly malignant? Likewise, it is not clear whether they are always progressive toward invasive carcinoma or there is any reversibility, and if it so, which factors favor it? It seems likely that the PanIN-2 and PanIN-3 represent committed lesions, but data about the dynamics are still frag-

mentary. *Brat et al*. for example, have reported that after appearance of atypical papillary hyperplasia (PanIN-2) 17 months to 10 years can elapse before development of infiltrative adenocarcinoma.[11] Because the PanIN terminology is relative new, many more studies are needed to clarify the exact biological nature of these ductal lesions.

Hormonal sensitivity

Although the ductal adenocarcinoma (that accounts for about 85% of all pancreatic cancers) is traditionally not regarded as a hormone responsive tumor, surprisingly, a great number of studies have provided evidence that it can be effectively influenced by natural and synthetic hormones. (Scattered neuroendocrine cells are consistently found in these tumors probably exerting a local paracrine effects, but unlikely to be malignant because they are absent in lymph node metastases.)[133] Cholecystokinin (CCK), secretin, bombesin, gastrin, EGF, TGF-α, insulin, IGF-1 or GH all exert a growth-promoting effect.[38] In the nitrosamine-induced hamster carcinogenesis model the gastrointestinal hormones (CCK, secretin) proved to be co-carcinogenic,[55,56] the growth of human pancreatic cancer xenografts could be significantly inhibited by the CCK-antagonist,[88] somatostatin-analog octreotide,[135] or could be accelerated by testosterone,[45] Treatment with LHRH-agonists or somatostatin-analogs resulted in a histologically proven regression of the hamster tumors,[149] mainly by inducing apoptosis.[127,128] Apoptosis-induction by octreotide has also been reported in human pancreatic cancer xenografts.[151] Tamoxifen and octreotide proved to be beneficial in both experimental and human studies.[101,118]

Among the hormones the role of *somatostatin* (SS) and its analogs seem to be the most controversial. Originally, this peptide came to light, because it was known to block the release of gastrointestinal hormones,[36] but it also became evident that SS antagonized the promoting effect of various growth factors, too. Recent discoveries revealed that the anti-neoplasic action of these hormones is rather complex: in addition to the beneficial effects mentioned before due to induction of apoptosis,[128,151] transient G0/G1 cell cycle block,[17] activation of the phosphotyrosine phosphatase,[78] and inhibition of the angiogenesis[95,152] should also be taken into consideration.[99]

Since the half-life of the native SS-peptide is several minutes in the circulation, long-acting analogs have been developed that retain the inhibiting properties of the mother hormone but resistant to the degradation. A great number of experimental and human studies have been performed using these analogs [RC-160 (Vapreotide, Octastatin); lanreotide (Somatuline); octreotide (Sandostatin)]. The basal or EGF-stimulated proliferation of various pancreatic cancer cell lines have been inhibited,[13,104,131] but different tumor types might respond differently.[101] Using a tumor-selective analog with no GH-release inhibiting activity (TT-232) over a 90% of inhibition was achieved in different pancreatic cancer cell lines inducing apoptotic cell death.[65,73] In the hamster-nitrosamine model of ductal adenocarcinoma many promising results have been published: SS-analogs (alone or in combination) prolonged the survival rate of the animals, decreased the tumorous pancreas weight, reduced the tumorous ascites and regressive histological changes and apoptosis were induced,[128,129,149,150] (*Figure 3a*) although unexpectedly, when *small doses* were administered, octreotide seemed even to promote pancreatic carcinogenesis.[49] This drug was also able to decrease the size and number of liver metastases in hamsters with chemically induced pancreatic cancer.[144] Given prophylactically, octreotide inhibited the development of the putative preneoplastic ductular lesions.[84] Various human pancreatic carcinomas (Mia-PaCa-2, CAV, SKI, etc.) growing as xenografts were also shown to be sensitive to hormonal manipulation with SS-analogs evidenced by significantly reduced tumor volume, tumor weight, growth rate, RNA–content, or by prolonged doubling time.[101,107,114,135] The increased apoptotic rate which was observed in animal carcinogenesis models has also been shown in xenografts: in PZX-5 carcinoma apoptosis induction was demonstrated by TUNEL-based Apoptag-immunohistochemistry and flow cytometry,[151] and it was also demonstrated that in PZX-15/F4 tumor the process of apoptosis was accompanied by a significant decrease of the intracellular phosphorylation state (*Figure 3b-d*).[148] TT-232 also inhibited tumor formation in human

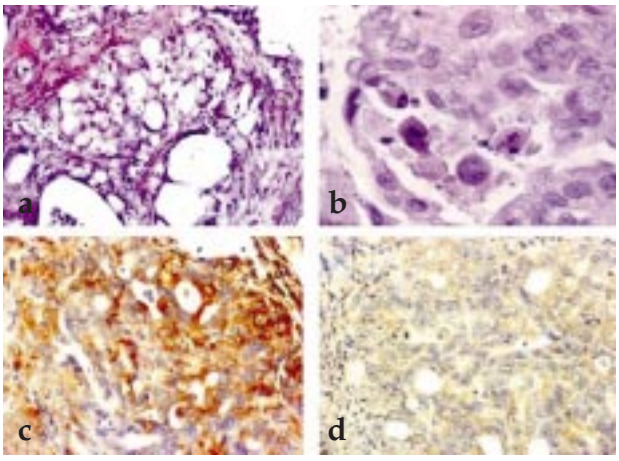

Figure 3. *Hormonal sensitivity of the pancreatic carcinoma.* ***(a)*** *Apoptotic cells in nitrosamine-induced hamster pancreatic cancer after treatment with somatostatin analog (HE, x100);* ***(b)*** *apoptotic activity in a human pancreatic adenocarcinoma xenograft (PZX-15/F4) after a 1-month treatment with octreotide (HE, x400);* ***(c-d)*** *immunohistochemical reactions in the same tumor without any treatment* ***(c)*** *or following Sandostatin-administration* ***(d)*** *(x200).*

pancreatic cancer xenograft model.[73] These and additional data have strongly indicated the hormonal responsiveness of this type of tumor.

Unfortunately, these suggestive results have not been reflected by clinical studies. SS-analogs administered as a monotherapy in advanced stage (less than 200 patients in the literature) yielded neither a complete remission nor a survival benefit even with megadoses, but in 15-20% of cases there was a significant improvement in the physical condition. Better results have been reported with combination treatments. While co-administration of octreotide and LHRH-analogs (despite the promising preclinical studies) did not result in a prolongation of the life,[35] combining tamoxifen and SS-analogs exhibited beneficial effects.

The treatment with this combination was preceded by promising pre-clinical studies demonstrating lower serum IGF-1 level and hepatic IGF-1 expression,[59] but tamoxifen alone has also been reported to induce a G0/G1 arrest accompanied by the increase in $p21^{WAF1}$ mRNA expression.[115] Clinical studies did reveal prolonged survival in non-operated[118] and in R0-resected patients.[143] Moreover, in addition to the longer median survival time (12 months vs. 3 months in a historical cohort) an important benefit was also observed: these patients did not require major analgesics until the final weeks of their illness. Tamoxifen can similarly improve the quality of life in pancreatic cancer patients when administered together with gemcitabine, and in these cases, partial response was achieved in 11% and 48% experienced stable disease.[134]

Although the above mentioned results do suggest the hormonal sensitivity of the ordinary pancreatic adenocarcinoma, an intriguing finding is the lack of surface somatostatin receptors (sstr) in them. Early receptor binding assay and phosphor autoradiography studies revealed specific receptors for SS on human pancreatic cancer cell lines or xenografts,[104,123,132] but later investigations have failed to identify their presence.[110,111] Similarly, none of 26 pancreatic adenocarcinomas proved to be positive using SS receptor scintigraphy,[30] and immunohistochemically, no sstr2A expression could be demonstrated either.[112] Although single or scattered clusters of chromogranin A and sstr2A immunoreactive cells were found in about half of the carcinomas investigated, but their proportion accounted just for about 5 to 10% of the entire tumorous cell population.[97] Despite these results, however, the somatostatin receptor genes are active in this tumors evidenced by the presence of mRNAs. In 1998 Fisher and coworkers have analyzed 11 adenocarcinomas and 9 human pancreatic cancer cell lines by using RT-PCR and they found that 7 of 9 cell lines and 8 of 11 tumors expressed SS receptor mRNAs for subtypes sstr1, 2, 5, but no sstr3 and 4. In several cell lines multiple receptor genes were active simultaneously. Contrary to these findings, however, functional surface receptors were lacking in all but one of the cases.[39] Somewhat similar observations have also been made by different groups.[13,14,67]

These results have raised the possibility that increasing the concentration of the *functional* cell surface SS receptors may render the pancreatic carcinomas sensitive to hormonal treatment. Several lines of experimental evidence suggest that gene transfer might offer a promising new strategy. *In vitro* studies revealed that correction of the sstr2 defect in pancreatic cancer cell lines by transfection with human SS receptor subtype-2 cDNA significantly reduced their clonogenicity in soft agar, inhibited the EGF-stimulated proliferation or decreased the viability of the cells.[24,47,67] Recent reports have also provided evidence that this method gives rise to a 4-5-fold apoptosis in transfected cells. Sstr2 gene transfer resulted not just in an enhanced apoptosis, but also sensitized tumor cells to TNFα or TRAIL-induced apoptosis, stimulated the executioner caspase activity, increased the basal and death ligand-induced caspase 8 cleaving and all these effects were accompanied by cytochrome C release into the cytosol.[47] Some *in vivo* studies also reinforce these findings. Transfected cells injected into nude mice displayed a suppressed tumor growth.[24] Intratumoral sstr2 gene transfer using recombinant adenovirus or synthetic carrier slowed down the tumorous progression both in primary and metastatic hamster carcinoma models, a significant decrease in PCNA labeling index was noted, and the percentage of apoptotic cells was increased by TUNEL in situ labeling method.[137] These beneficial effects were seen despite the fact that only small percentage of tumor cells (cc. 2%) had been transfected.

The results of *Fueger et al*. are also worth mentioning because they have a clinical impact. In different pancreatic cancer cell lines (BxPc-3, Panc-1, ASPC-1, Capan-1) expressing various SS receptors gemcitabine reversibly reduced the high- and low affinity binding sites at a dose dependent manner. After removing the cytostatic drug it was followed by significant or extremely high (depending on the cell lines used) overexpression of binding sites within several days after treatment.[40] Similarly, increased expression of receptors was shown after cisplatin administration, but without the early downregulation effect. These finding further reinforce the potential applicability of somatostatin-analogs in the treatment of pancreatic cancer.

Among the gastrointestinal hormones, *gastrin* is one of the well known promoter of the pancreatic carcinogenesis, therefore, this hormone has also become a novel target in the fight against the pancreatic cancer. Experimental studies have revealed that anti-gastrin oligonucleotides result in an 88% of cell growth inhibition *in vitro*, and the human BxPc-3 tumor grown as xenograft in nude mice was found to be inhibited upon intratumoral administration of these compound.[124] Human studies have also been performed

using an anti-gastrin immunogen product, G17DT (Gastrimmune). This vaccine is composed of diphtheria toxoid serving as a carrier attached with a synthetic peptide analogous to gastrin-17. G17DT was recently granted an orphan drug status in US, Australia and in Europe for the treatment of pancreatic or gastric cancer. Administration of this compound in patients with advanced pancreatic cancer yielded antibody response to gastrin in 67% accompanied by survival prolongation (217 days for antibody responders vs. 121 days for nonresponders).[12]

Although the above mentioned results are still too premature to draw a final conclusion, they show further promise for the hormonal sensitivity of the pancreatic cancer and it is worth seeking new frontiers in this field of oncology.

Mixed exocrine-endocrine carcinomas

The pancreatic cancer relative frequently contains endocrine (chromogranin A positive and/or polypeptide hormone containing neuroendocrine) cells among the malignant ductal cells,[146] however, the mixed ductal-endocrine carcinomas are clearly separated by the WHO classification, because in this tumor the two components are intimately admixed with the endocrine element being comprised of at least one-third of the whole tumorous population. (*Figure 4*.) These malignant neoplasms are rather rare, accounting about 1% of the all pancreatic cancer cases and therefore, our knowledge about them is still limited. (Acinar-endocrine carcinomas represent a real curiosity: about 20 well documented cases have been reported to date, and only a single paper is known describing a triphasic – ductal/acinar/islet cell tumor.)[92] Follow-up studies showed that the biological behavior was principally dictated by the exocrine part, and the survival is as poor as in the ordinary adenocarcinoma patients.

An interesting question about this tumor is its histogenesis, also from the point of view of the interrelationship between the exocrine and endocrine cells. The pancreas develops from the dorsal and ventral endodermal buds that will later fuse. Pancreas duodenum homeodomain protein (Pdx-1) gene is essential for the bud expansion initiating pancreatic differentiation but it is not sufficient for completing it. In other words, all specific pancreatic cells (ducts, acini, islets) are derived from pdx-1 expressing progenitors, that morphologically resemble primitive duct-like structures but biologically they are uncommitted. Further formation of exocrine and endocrine structures is governed by different transcription factors: upon the effect of *pd48* the pancreatic cells undergo maturation toward ductal and acinar elements, while *Pax6*, *Isl1*, *ngn3*, *NeuroD* genes result in an endocrine but still multidirectional cell population requiring activation of different other genes to be committed to specific (α, β, δ) lineages.[44] Thus, in the adult pancreas the secretory and hormone-producing components are separated and functioning under control of different genes. How do these cells produce mixed tumors?

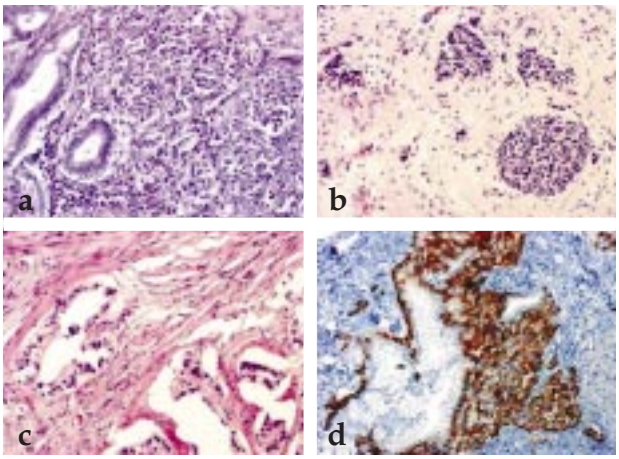

Figure 4. *Mixed ductal-endocrine carcinoma.* ***(a)*** *intermingled exocrine and endocrine elements in the same tumor (HE, x100);* ***(b)*** *one part of the tumor is composed of clusters of proliferative endocrine cells (HE, x100);* ***(c)*** *antoher part of the same tumor showing an infiltrative adenocarcinoma component (HE, x100);* ***(d)*** *in the tumor bidirectional (ductal and endocrine) differentiation is seen (chromogranin A immunohistochemistry, x100).*

Several studies provided evidence that the separated compartments in pancreas did not represent terminally differentiated cell types, and under specific circumstances transition could occur. The least stable cells seem to be the acini: when cultured in vitro, within several days they transdifferentiate to ductal phenotype cells without dividing,[51] and this process is accompanied by reinduction of PDX-1 resulting in cells showing similar characteristics to precursor cells.[117] New islet cells (especially insulin-producing ones) can also be formed after tissue injury and this process seems to involve the duct-like exocrine cells which can differentiate toward hormone-expressing cells.[9] In turn, islets are also able to "retrodifferentiatie" as it is indicated by in vitro studies: when islets were maintained in culture for more than a year, the endocrine cells gradually underwent to ductal, acinar or intermediary cells, later all the cells were replaced by multipotential, undifferentiated cells.[121] This process requires cAMP-mediated signal transduction and appropriate integrin-matrix interaction.[140]

Abnormal differentiation of islets in pancreatic cancer has also been reported: *Pour at al*. have observed that in 25 of 37 adenocarcinoma tissues the Langerhans islands showed expression of tumor-associated antigens such as CA19-9, Du-PAN2 or TAG-72 suggesting transformation of antigen expressing islet cells to ductal structures.[102]

Although the developmental mechanisms in mixed exocrine-endocrine pancreatic tumors are still obscure, but

these indirect data might suggest that the process could start from either the exocrine or the islet cells: carcinogenic effects may result in a transdifferentiation to progenitor cells retaining capacity of expressing both endocrine and exocrine phenotype.[64] Unfortunately, data are lacking about the expression of pdx-1, p48, ngn3, Pax6, or Isl1 in these neoplasms that could reinforce the hypothesis.

Chemoprevention

Chemopreventive compounds could offer a promise of reducing cancer risk through supplementation in the human diet. In past decades, several large-scale clinical trials have been undertaken to study cancer prevention in the breast, skin, lung, colon or prostate,[77] but only limited information is available regarding the pancreatic malignancies. Investigation, however, would be desirable especially in family members where first-degree relatives have been diagnosed with this tumor, or in people displaying and increased risk (e.g. heavy smokers). *In vitro* or *in vivo* experimental studies have demonstrated that the concept of chemoprevention can also be applicable to the pancreatic cancer, too.

As early as the end of the 1980s, it was reported that dietary supplementation with *retinoids* inhibited the progression of the asaserine–initiated pancreatic carcinogenesis in rats and this effect was further enhanced by selenium.[21] Similar observations were also made in long–term experiments.[145] Beta- (but not alpha-) carotene feeding was also found to decrease the number of ductal lesions in Syrian hamsters treated with chemical carcinogens.[82] In vitro studies have also indicated a beneficial effect: retinoids caused a decreased bcl2/bax ratio,[96] or inhibited the growth of human pancreatic cancer cell lines alone or in combination with vitamin D analogs.[153] Based on these findings clinical trials have also been conducted, but they yielded no spectacular results. In a phase II study α-interferon was combined with retinoid acid in patients with advanced stage of this tumor resulting in a prolonged stable disease in about two-third of cases.[113] Another combination treatments have been performed by Italian authors administering chemotherapy + β-interferon + retinoids in metastatic pancreatic cancer cases. They could not achieve dramatic survival benefit, moreover, the complex treatment was limited by the high toxicity rate, some of the side effects being rather severe.[109] *Rautalahti et al*. have reported the results of Alpha-Tocopherol Beta-Carotene Cancer Prevention Study: there were more than 29 000 male smoker participants whose diet was supplemented with 50 mg/day dl-alpha-tocopherol and 20 mg/day β-carotene for 5-8 years. Unfortunately, both supplementations were statistically nonsignificant, although somewhat less pancreatic cancers developed in patients receiving β-carotene than in the matching group.[108] Summing up, to date therefore, retinoids do not seem to offer a chemopreventive effect for pancreatic cancer.

A similarly controversial issue is the significance of the food-derived *polyphenols*. In vitro studies have revealed that black and green tea extracts strongly inhibited the pancreatic cancer cell growth.[80] Quercetin or resveratrol inhibited the cell proliferation, accompanied by enhanced apoptosis, mitochondrial depolarization, cytochrome c release and caspase-3 activation.[26,89] Positive results were also seen in *in vivo* experiments: polyphenols significantly decreased the process of ductal carcinogenesis in Syrian hamsters,[82] or inhibited the growth of primary tumor and prevented metastasis formation in nude mice model.[89] Some human studies, however, do not entitle us to draw any conclusion: a population-based case-control study in Shanghai have indicated that regular green tea drinking lowers the risk of colorectal and pancreatic cancer in both sexes,[63] but in an American prospective cohort study of about 34 000 postmenopausal women it was found that the tea intake was not related to pancreatic cancer incidence.[52]

Cyclooxygenase-2 (COX-2), a key enzyme of the prostaglandin synthesis has strongly been implicated in the carcinogenesis of the gastrointestinal tract and many well documented observations support the potential chemopreventive effect of COX-2 inhibitors in colorectal cancer. Much less is known about the role of cyclooxygenase in pancreatic cancer, albeit, promising experimental data are being accumulated.[27] COX-2 mRNA levels are elevated in most of the tumors, and the enzyme is frequently (over 60%) up-regulated.[29,72,87] Immunohistochemical studies have revealed that the average percentage of positive cells in human pancreatic carcinoma was 47% as compared with 19% found in normal duct, and the expression increased from normal to PanIN to adenocarcinoma.[81] Different COX-2 blockers have led to increased apoptosis in vitro,[28,29] produced a dose-dependent inhibition of cell proliferation[87] and the inhibitory effect was found to be correlated with the degree of immunohistochemical expression[72]. COX-2 inhibitors were also reported to potentiate the antiproliferative effect of gemcitabine.[147] However, some nonsteroidal anti-inflammatory drugs (NSAIDs) may be antimitotic in pancreatic cancer cells but not necessarily via the cyclooxygenase route.[29]

Despite these encouraging experimental results, human investigations about the applicability of NSAIDs are sparse and inconclusive. *Kokawa et al*. have reported that 57% of human pancreatic cancer samples expressed COX-2 immunohistochemically, but no correlation was found with clinicopathologic indices.[72] Prospective studies in USA have shown that among the women with regular use of aspirin there was a trend to decreasing risk of pancreatic cancer incidence. Interestingly enough, however, other NSAIDs were not associated with incident pancreatic carcinoma.[4] Unfortunately, hospital-based case-control studies could not reinforce the risk-lowering effect of the aspirin.[85]

Concluding remarks

Despite several lines of tempting ideas and promising experimental results there is no breakthough in the understanding of pancreatic cancer; in the clinical practice the diagnosis of this tumor still anticipates a grim prognosis. Unfortunately, an intriguing discrepancy exists between the in vitro or animal studies and the human experience which cannot be explained simply by the interspecies differences, because the positive findings in xenograft systems (e.g. *human* tumors transplanted into immunosuppressed animals) are not regularly reinforced by the clinical trials either. Contrary to the traditional view, the pancreatic cancer seems to be sensitive and responsive to hormonal effects, but the increased apoptotic activity does not lead to clinically relevant tumor-inhibiting responses. Gene therapy and the chemoprevention are still at an early stage. Pancreatic cancer has remained a continuing challenge for oncology.

References

1. *Ahmad NA, Lewis JD, Ginsberg GG, et al:* Long term survival after pancreatic resection for pancreatic adenocarcinoma. Am J Gastroenterol 96: 2609-2615, 2001
2. *Alanen KA, Joensuu:* Long-term survival after pancreatic adenocarcinoma – often a misdiagnosis? Br J Cancer 68: 1004-1005, 1993
3. *Anderson KE, Hammons GJ, Kadlubar FF, et al:* Metabolic activation of aromatic amines by human pancreas. Carcinogenesis 18: 1085-1092, 1997
4. *Anderson KE, Johnson TW, Lazovich D, et al:* Association between nonsteroidal anti-inflammatory drug use and the incidence of pancreatic cancer. J Natl Cancer Inst 94: 1168-1171, 2002
5. *Anderson KE, Sinha R, Kulldorff M, et al:* Meat intake and cooking techniques: associations with pancreatic cancer. Mutat Res 30: 225-231, 2002
6. *Anttila A, Pukkala E, Sallmen M, et al:* Cancer incidence among Finnish workers exposed to halogenated hydrocarbons. J Occup Environ Med 37: 797-806, 1995
7. *Arundel SE, Kinnier-Wilson LM:* Parental occupations and cancer: a review of the literature. J Epidemiol Community Health 40: 30-36, 1986
8. *Biankin AV, Kench JG, Morey AL, et al:* Overexpression of p21 (WAF1/CIP1) is an early event in the development of pancreatic intraepithelial neoplasia. Cancer Res 61: 8830-8837, 2001
9. *Bouwens L:* Transdifferentiation versus stem cell hypothesis for the regeneration of islet beta-cells in the pancreas. Microsc Res Tech 43: 332-336, 1998
10. *Boyle P, Maisonneuve P, Bueno de Mesquita B, et al:* Cigarette smoking and pancreas cancer: a case control study of the search programme of the IARC. Int J Cancer 67: 63-71, 1996
11. *Brat DJ, Lillemoe KD, Yeo CJ, et al:* Progression of pancreatic intraductal neoplasias to infiltrating adenocarcinoma of the pancreas. Am J Surg Pathol 22: 163-169, 1998
12. *Brett BT, Smith JC, Bouvier CV, et al:* Phase II study of antigastrin-17 antibodies, raised to G17DT, in advanced pancreatic cancer. J Clin Oncol 20: 4225-4235, 2002
13. *Burghardt B, Barabás K, Marcsek Z, et al:* Inhibitory effect of a long-acting somatostatin analogue on EGF-stimulated cell proliferation in Capan-2 cells. J Physiol Paris 94: 57-62, 2000
14. *Buscail L, Saint-Laurent N, Chastre E, et al:* Loss of ss2 somatostatin receptor gene expression in human pancreatic and colorectal cancer. Cancer Res 56: 1823-1827, 1996
15. *Cantor KP, Silberman W:* Mortality among aerial pesticide applicators and flight instructors: follow-up from 1965-1988. Am J Ind Med 36: 239-247, 1999
16. *Chen J, Baithun SI, Ramsay MA:* Histogenesis of pancreatic carcinomas: a study based on 248 cases. J Pathol 146: 65-76, 1985
17. *Cheung NW, Boyages SC:* Somatostatin-14 and its analog octreotide exert a cytostatic effect on GH3 rat pituitary tumor cell proliferation via a transient G0/G1 cell cycle block. Endocrinology, 136: 4174-4181, 1995
18. *Clary T, Ritz B:* Pancreatic cancer mortality and organochlorine pesticide exposure in California, 1989-1996. Am J Ind Med 43: 306-313, 2003
19. *Cottliar AS, Fundia AF, Moran C, et al:* Evidence of chromosome instability in chronic pancreatitis. J Exp Clin Cancer 19: 513-517, 2000
20. *Cubilla AL, Fitzgerald PJ:* Morphological lesions associated with human primary invasive nonendocrine pancreas cancer. Cancer Res 36: 2690-2698, 1976
21. *Curphey TJ, Kuhlmann ET, Roebuck BD, et al:* Inhibition of pancreatic and liver carcinogenesis in rats by retinoid- and selenium-supplemented diets. Pancreas 3: 36-40, 1988
22. *Day JD, Digiuseppe JA, Yeo C, et al:* Immunohistochemical evaluation of HER-2/neu expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasms. Hum Pathol 27: 119-124, 1996
23. *De Gonzalez AB, Sweetland S, Spencer E:* A meta-analysis of obesity and the risk of pancreatic cancer. Br J Cancer 89: 519-523, 2003
24. *Delesque N, Buscail L, Estève JP, et al:* Sst2 somatostatin receptor expression reverses tumorigenicity of human pancreatic cancer cells. Cancer Res 57: 956-962, 1997
25. *Dell L, Teta MJ:* Mortality among workers at a plastics manufacturing and research and development facility: 1946-1988. Am J Ind Med 28: 373-384, 1995
26. *Ding XZ, Adrian TE:* Resveratrol inhibits proliferation and induces apoptosis in human pancreatic cancer cells. Pancreas 25: 71-76, 2002
27. *Ding XZ, Hennig R, Adrian TE:* Lipoxygenase and cyclooxygenase metabolism: new insights in treatment and chemoprevention of pancreatic cancer. Mol Cancer 2: 1-10, 2003
28. *Ding XZ, Tong WG, Adrian TE:* Blockade of cycloxygenase-2 inhibits proliferation and induces apoptosis in human pancreatic cancer cells. Anticancer Res 20: 2525-2631, 2000
29. *Eibl G, Reber HA, Wente MN, et al:* The selective cyclooxygenase-2 inhibitor nimesulide induces apoptosis in pancreatic cancer cells independent of COX-2. Pancreas 26: 33-41, 2003
30. *vanEijck CHJ, Lamberts SWJ, Lemaire LCJ, at al:* The use of somatostatin receptor scintigraphy in the differential diagnosis of pancreatic duct cancers and islet cell tumors. Ann Surg 224: 119-124, 1996
31. *Ekbom A, McLaughilin JK, Karlsson BM,et al:* Pancreatitis and pancreatic cancer. A population-based study. J Natl Cancer Inst 86: 625-627, 1994
32. *Engeland A:* Trends in the incidence of smoking-associated cancers in Norway, 1954-93. Int J Cancer 68: 39-46, 1996
33. *Ewing J:* Neoplastic diseases. A treatise on tumors. 3rd ed., W.B. Saunders, Philadelphia, London, 1928
34. *Faivre J, Forman D, Esteve J, et al:* Survival of patients with primary liver cancer, pancreatic cancer and biliary tract cancer in Europe. Eur J Cancer 34: 2184-2190, 1998

35. *Fazeny B, Baur M, Prohaska M, et al:* Octreotide combined with goserelin in the therapy of advanced pancreatic cancer – results of a pilot study and review of the literature. J Cancer Res Clin Oncol 123: 45-52, 1997
36. *Fenoglio CM, King DW:* Somatostatin: an update. Human Pathol 14: 475-479, 1983
37. *Fernandez E, La Vecchia C, Porta M, et al:* Trends in pancreatic cancer mortality in Europe, 1955-1989. Int J Cancer 57: 786-792, 1994
38. *Fisher WE, Muscarella P, Boros LG, et al:* Gastrointestinal hormones as potential adjuvant treatment of exocrine pancreatic adenocarcinoma. Int J Pancreatol 24: 169-180, 1998
39. *Fisher WE, Doran TA, Muscarella P, et al:* Expression of somatostatin receptor subtype 1-5 genes in human pancreatic cancer. J Natl Cancer Inst 90: 322-324, 1998
40. *Fueger BJ, Hamilton G, Raderer M, et al:* Effects of chemotherapeutic agents on expression of somatostatin receptors in pancreatic tumor cells. J Nucl Med 42: 1856-1862, 2001
41. *Garabrant DH, Held J, Langholz B, et al:* DDT and related compounds and risk of pancreatic cancer. J Natl Cancer Inst 84: 764-771, 1992
42. *Ghadirian P, Boyle P, Simard A, et al:* Reported family aggregation of pancreatic cancer within a population-based case-control study in the Francophone community in Montreal, Canada. Int J Pancreatol 10: 183-196, 1991
43. *Ginsberg GG:* New developments in pancreatic cancer. Semin Gastrointest Dis 11: 162-167, 2000
44. *Grapin-Botton A, Majithia AR, Melton DA:* Key events of pancreas formation are triggered in gut endoderm by ectopic expression of pancreatic regulatory genes. Gen Dev 15: 444-454, 2001
45. *Greenway B, Iqbal MJ, Johnson PJ, et al:* Oestrogen receptor proteins in malignant and fetal pancreas. Br Med J 283: 751-753, 1981
46. *Gudjonsson B:* Survival statistics gone awry: pancreatic cancer, a case in point. J Clin Gastroenterol 35: 180-184, 2002
47. *Guillermet J, Saint-Lauren N, Rochaix P, et al:* Somatostatin receptor subtype 2 sensitizes human pancreatic cancer cells to death ligand-induced apoptosis. Proc Natl Acad Sci USA 100, 155-160, 2003
48. *Gullo L:* Risk of pancreatic and periampullary cancer following cholecystectomy. Ann Oncol 10: (Suppl 4), 127-128, 1999
49. *Haddock G, Harrison DJ, Carter DC:* The effect of the somatostatin analogue SMS 201-995 on experimental pancreatic carcinogenesis in the Syrian golden hamster. Carcinogenesis 12: 1103-1107, 1991
50. *Hahn SA, Greenhalf B, Ellis I, et al:* BRCA2 germline mutations in familial pancreatic carcinoma. J Natl Cancer Inst 95: 214-221, 2003
51. *Hall PA, Lemoine NR:* Rapid acinar to ductal transdifferentiation in cultured human exocrine pancreas. J Pathol 166: 97-103, 1992
52. *Harnack LJ, Anderson KE, Zheng W, et al:* Smoking, alcohol, coffee, and tea intake and incidence of cancer of the exocrine pancreas: the Iowa Women's Health Study. Cancer Epidemiol Biomarkers Prev 6: 1081-1086, 1997
53. *Hecht SS, Hoffmann D:* N-nitroso compounds and tobacco-induced cancers in man. IARC Sci Publ 105:54-61, 1991
54. *Hemminki K, Li X:* Familial and second primary pancreatic cancers: a nationwide epidemiologic study from Sweden. Int J Cancer 103: 525-530, 2003
55. *Howatson AG, Carter DC:* Pancreatic carcinogenesis – enhancement by cholecystokinin in the hamster-nitrosamine model. Br J Cancer 51: 107-114, 1985
56. *Howatson AG, Carter DC:* Pancreatic carcinogenesis: effect of secretin in the hamster-nitrosamine model. J Natl Cancer Inst 78: 101-105, 1987
57. *Hruban RH, Adsay NV, Albores-Saavedra J, Compton C, et al:* Pancreatic intraepithelial neoplasia. A new nomeclature and classification system for pancreatic duct lesions. Am J Surg Pathol 25: 579-586, 2001
58. *Hruban RH, Canto MI, Yeo CJ:* Prevention of pancreatic cancer and strategies for management of familial pancreatic cancer. Dig Dis 19: 76-84, 2001
59. *Huynh H, Pollak M:* Enhancement of tamoxifen-induced suppression of insulin-like growth factor 1 gene expression and serum level by a somatostatin analogue. Biochem Biophys Res Comm 203: 253-259, 1994
60. *Imaizumi Y:* Longitudinal Gompertzian analysis of mortality from pancreatic cancer in Japan, 1955-1993. Mech Ageing Dev 90: 163-181, 1996
61. *Janes RH, Niederhuber JE, Chmiel JS, et al:* National patterns of care for pancreatic cancer. Results of a survey by the Commission on Cancer. Ann Surg 223: 261-272, 1996
62. *Jemal A, Murray T, Samuels A,, et al:* Cancer statistics, 2003. CA Cancer J Clin 53: 5-26, 2003
63. *Ji BT, Chow WH, Hsing AW, et al:* Green tea consumption and the risk of pancreatic and colorectal cancers. Int J Cancer 27: 255-258, 1997
64. *Kamisawa T, Tu Y, Egawa N, et al:* Ductal and acinar differentiation in pancreatic endocrine tumors. Dig Dis Sci 47: 2254-2261, 2002
65. *Kéri G, Érchegyi A, Horváth A, et al:* A tumor-selective somatostatin analog (TT-232) with strong *in vitro* and *in vivo* antitumor activity. Proc Natl Acad Sci USA 93: 12513-12518, 1996
66. *Kernan GJ, Ji BT, Dosemeci M, et al:* Occupational risk factors for pancreatic cancer: a case-control study based on death certificates from 24 U.S. states. Am J Ind Med 36: 260-270, 1999
67. *Kikutsuji T, Harada M, Tashiro S, et al:* Expression of somatostatin receptor subtypes and growth inhibition in human exocrine pancreatic cancer. J Hepatobiliary Pancreat Surg 7: 496-503, 2000
68. *Klein AP, Beaty TH, Bailey-Wilson JE, et al:* Evidence for a major gene influencing risk of pancreatic cancer. Genet Epidemiol 23: 133-149, 2002
69. *Klein AP, Hruban RH, Brune KA, et al:* Familial pancreatic cancer. Cancer J 7: 266-273, 2001
70. *Klein WM, Hruban RH, Klein-Szanto AJ, et al:* Direct correlation between proliferative activity and dysplasia in pancreatic intraepithelial neoplasia (PanIN): additional evidence for a recently proposed model of progression. Modern Pathol 15: 441-447, 2002
71. *Klimstra DS, Longnecker DS:* K-ras mutations in pancreatic ductal proliferative lesions. Am J Pathol 145: 1547-1548, 1994
72. *Kokawa A, Kondo H, Gotoda T, et al:* Increased expression of cyclooxygenase-2 in human pancreatic neoplasms and potential for chemoprevention by cyclooxygenase inhibitors. Cancer 91: 333-338, 2001
73. *Lee JU, Hosotani R, Wada M, et al:* Antiproliferative activity induced by the somatostatin analogue, TT-232, in human pancreatic cancer cells. Eur J Cancer 38: 1526-1534, 2002
74. *Levi F, Decarli A, La Vecchia C:* Trends in cancer mortality in Switzerland, 1951-1984. Rev Epidemiol Sante Publique 36: 15-25, 1988
75. *Lillemoe KD, Yeo CJ, Cameron JL:* Pancreatic cancer: state-of-the-art care. CA Cancer J Clin 50: 241-268, 2000
76. *Lin Y, Tamakoshi A, Kawamura T, Inaba Y, et al:* Risk of pancreatic cancer in relation to alcohol drinking, coffee consumption and

medical history: findings from the Japan collaborative cohort study for evaluation of cancer risk. Int J Cancer 99: 742-746, 2002
77. *Lippman SM, Lee JJ, Sabichi AL:* Cancer chemoprevention: progress and promise. J Natl Cancer Inst 90: 1514-1528, 1998
78. *Lopez F, Esteve JP, Buscail L, et al:* Molecular mechanisms of antiproliferative effect of somatostatin: involvement of a tyrosine phosphatase. Metabolism, 45: (Suppl. 1.) 14-16, 1996
79. *Lüttges J, Diederichs A, Menke MA, et al:* Ductal lesions in patients with chronic pancreatitis show K-ras mutations in a frequency similar to that in the normal pancreas and lack nuclear immunoreactivity for p53. Cancer 88: 2495-2504, 2000
80. *Lyn-Cook BD, Rogers T, Yan Y, et al:* Chemopreventive effects of tea extracts and various components on human pancreatic and prostate tumor cells in vitro. Nutr Cancer 35: 80-86, 1999
81. *Maitra A, Ashfaq R, Gunn CR, et al:* Cyclooxygenase 2 expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasia: an immunohistochemical analysis with automated cellular imaging. Am J Clin Pathol 118: 194-201, 2002
82. *Majima T, Tsutsumi M, Nishino H, et al:* Inhibitory effects of beta-carotene, palm carotene, and green tea polyphenols on pancreatic carcinogenesis initiated by N-nitrosobis(2-oxopropyl) amine in Syrian golden hamsters. Pancreas 16: 13-18, 1998
83. *Manes G, Balzano A, Vaira D:* Helicobacter pylori and pancreatic disease. J Pancreas (Online) 4: 111-116, 2003
84. *Meijers M, Woutersen RA, van Garderen-Hoetmer A, et al:* Effects of sandostatin and castration on pancreatic carcinogenesis in rats and hamsters. Int J Cancer 50: 246-251, 1992
85. *Menezes RJ, Huber KR, Mahoney MC, et al:* Regular use of aspirin and pancreatic cancer risk. BMC Public Health 2: 1-18, 2002
86. *Michaud DS, Giovannucci E, Willett WC, et al:* Coffee and alcohol consumption and the risk of pancreatic cancer in two prospective United States cohorts. Cancer Epidemiol Biomarkers Prev 10: 429-437, 2001
87. *Molina MA, Sitja-Arnau M, Lemoine MG, et al:* Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines: growth inhibition by nonsteroidal anti-inflammatory drugs. Cancer Res 59: 4356-4362, 1999
88. *Morimoto H, Nio Y, Tsubono M, et al:* Inhibitory effects of cholecystokinin antagonist, loxiglumide (CR-1505), on the growth of freshly separated and xenografted human pancreatic cancer. J Surg Oncol 53: 47-53, 1993
89. *Mouria M, Gukovskaya AS, Jung Y, et al:* Food-derived polyphenols inhibit pancreatic cancer growth through mitochondrial cytochrome C release and apoptosis. Int J Cancer 98: 761-769, 2002
90. *Mulligan NJ, Yang S, Andry C, et al:* The role of p21ras in pancreatic neoplasia and chronic pancreatitis. Hum Pathol 30: 602-610, 1999
91. *Nilsson HO, Stenram U, Ihse I, et al:* Re: Helicobacter pylori seropositivity as a risk factor for pancreatic cancer. J Natl Cancer Inst 94: 632-633, 2002
92. *Okada Y, Mori H, Tsutsumi A:* Duct-acinar-islet cell tumor of the pancreas. Pathol Int 45: 669-676, 1995
93. *Orth M, Gansauge F, Gansauge S, et al:* K-ras mutations at codon 12 are rare events in chronic pancreatitis. Digestion 59: 120-124, 1998
94. *Parkash O:* Carcinoma of pancreas. A study based on autopsy data from 1928 to 1972 in Vienna, Austria. Digestion 13: 137-144, 1975
95. *Patel PC, Barrie R, Hill N, et al:* Postreceptor signal transduction mechanisms involved in octreotide-induced inhibition of angiogenesis. Surgery 116: 1148-1152, 1994
96. *Pettersson F, Dalgleish AG, Bissonnette RP, et al:* Retinoids cause apoptosis in pancreatic cancer cells via activation of RAR-gamma and altered expression of Bcl2/Nax. Br J Cancer 87: 555-561, 2002
97. *Pilichowska M, Kimura N, Schindler M, et al:* Somatostatin type 2A receptor immunoreactivity in human pancreatic adenocarcinomas. Endocrine Pathol 12: 147-155, 2001
98. *Pleško I, Dimitrova E, Somogyi J, et al:* Geographic patterns and trends of liver, biliary tract and pancreas cancer mortality in Slovakia. Neoplasma 30: 97-104, 1983
99. *Pollak MN, Schally AV:* Mechanisms of antineoplastic action of somatostatin analogs. Proc Soc Exp Biol Med 217: 143-152, 1998
100. *Popiela T, Kedra B, Karcz D, et al:* Long-term results of pancreatic cancer surgery. Przegl Lek 57: (Suppl. 5) 22-26, 2000
101. *Poston GJ, Townsend CM jr, Rajaraman S, et al:* Effect of somatostatin and tamoxifen on the growth of human pancreatic cancer in nude mice. Pancreas 5: 151-157, 1990
102. *Pour PM, Schmied BM, Ulrich AB, et al:* Abnormal differentiation of islet cells in pancreatic cancer. Pancreatology 1: 110-116, 2001
103. *Prokopczyk B, Hoffmann D, Bologna M, et al:* Identification of tobacco-derived compounds in human pancreatic juice. Chem Res Toxicol 15: 677-85, 2002
104. *Qin Y, Ertl T, Groot K, et al:* Somatostatin analog RC-160 inhibits growth of CFPAC-1 human pancreatic cancer cells in vitro and intracellular production of cyclic adenosine monophosphate. Int J Cancer 60: 694-700, 1995
105. *Queneau PE, Adessi GL, Thibault P, et al:* Early detection of pancreatic cancer in patients with chronic pancreatitis: diagnostic utility of a K-ras point mutation in the pancreatic juice. Am J Gastroenterol 96: 700-704, 2001
106. *Raderer M, Wrba F, Kornek G, et al:* Association between Helicobacter pylori infection and pancreatic cancer. Oncology 55: 16-19, 1998
107. *Radulovic S, Comaru-Schally AM, Milovanovic S, et al:* Somatostatin analogue RC-160 and LH-RH antagonist SB-75 inhibit growth of MIA PaCa-2 human pancreatic cancer xenografts in nude mice. Pancreas 8: 88-97, 1993
108. *Rautalahti MT, Virtamo JR, Taylor PR, et al:* The effects of supplementation with alpha-tocopherol and beta-carotene on the incidence and mortality of carcinoma of the pancreas in a randomized, controlled trial. Cancer 86: 37-42, 1999
109. *Recchia F, Sica G, Casucci D, et al:* Advanced carcinoma of the pancreas: phase II study of combined chemotherapy, beta-interferon, and retinoids. Am J Clin Oncol 21: 275-278, 1998
110. *Reubi JC:* Relevance of somatostatin receptors and other peptide receptors in pathology. Endocrine Pathol 8: 11-20, 1997
111. *Reubi JC, Horisberger U, Essed EC, et al:* Absence of somatostatin receptors in human exocrine pancreatic adenocarcinomas. Gastroenterology 95: 760-763, 1988
112. *Reubi JC, Kappeler A, Waser B, et al:* Immunohistochemical localization of somatostatin receptors sst2A in human tumors. Am J Pathol 153: 233-245, 1998
113. *Riecken EO, Rosewicz S:* Retinoids in pancreatic cancer. Ann Oncol 10: Suppl. 4, 197-200, 1999
114. *Robertson JF, Watson SA, Hardcastle JD:* Effect of gastrointestinal hormones and synthetic analogues on the growth of pancreatic cancer. Int J Cancer 63: 69-75, 1995
115. *Robinson EK, Grau AM, Evans DB, et al:* Cell cycle regulation of human pancreatic cancer by tamoxifen. Ann Surg Oncol 5: 342-349, 1998

116. *Rohan T, McMichael AZ:* Alimentary tract cancer mortality in Australia, 1908-1978. An epidemiological appraisal. Med J Aust 1: 232-235, 1981
117. *Rooman L, Heremans Y, Heumberg H, et al:* Modulation of rat pancreatic acinoductal transdifferentiation and expression of PDX-1 in vitro. Diabetologia 43: 907-914, 2000
118. *Rosenberg, L, Barkun AN, Denis MH, et al:* Low dose octreotide and tamoxifen in the treatment of adenocarcinoma of the pancreas. Cancer 75: 23-28, 1995
119. *Rulyak SJ, Lowenfels AB, Maisonneuve P, et al:* Risk factors for the development of pancreatic cancer in familial pancreatic cancer kindreds. Gastroenterology 124: 1292-1299, 2003
120. *Schlosser W, Schoenberg MH, Rhein E, et al:* Pankreaskarzinom bei chronischer Pankreatitis mit entzündlichem Pankreaskopftumor. Z Gastroenterol 34: 3-8, 1996
121. *Schmied BM, Ulrich A, Matsuzaki H, et al:* Transdifferentiation of human islet cells in a long-term culture. Pancreas 23: 157-171, 2001
122. *Selenskas S, Teta MJ, Vitale JN:* Pancreatic cancer among workers processing synthetic resins. Am J Ind Med 28: 385-98, 1995
123. *Singh P, Townsend CM jr, Poston GJ, et al:* Specific binding of cholecystokinin. estradiol and somatostatin to human pancreatic cancer xenografts. J Steroid Biochem Mol Biol 39: 759-767, 1991
124. *Smith JP, Verderame MF, Zagon IS:* Antisense oligonucleotides to gastrin inhibit growth of human pancreatic cancer. Cancer Lett 135: 107-112, 1999
125. *Stolzenberg-Solomon RZ, Blaser MJ, Limburg PJ, et al:* Helicobacter pylori seropositivity as a risk factor for pancreatic cancer. J Natl Cancer Inst 93: 937-941, 2001
126. *Su GH, Hruban RH, Bansal RK, Bova GS, et al:* Germline and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic and biliary cancers. Am J Pathol 154: 1835-1840, 1999
127. *Szende B, Srkalovic G, Schally AV, et al:* Inhibitory effects of analogs of luteinizing hormone-releasing hormone and somatostatin on pancreatic cancers in hamsters. Events that accompany regression. Cancer 65: 2279-2290, 1990
128. *Szende B, Zalatnai A, Schally AV:* Programmed cell death (apoptosis) in pancreatic cancers of hamsters after treatment with analogs of both luteinizing hormone-releasing hormone and somatostatin. Proc Natl Acad Sci USA 86: 1643-1647, 1989
129. *Szepesházi K, Lapis K, Schally, AV:* Effect of combination treatment with analogs of luteinizing hormone-releasing hormone (LH-RH) or somatostatin and 5-fluorouracil on pancreatic cancer in hamsters, Int J Cancer 49: 260-266, 1991
130. *Tabata T, Fujimori T, Maeda S, et al:* The role of Ras mutation in pancreatic cancer, precancerous lesions, and chronic pancreatitis. Int J Pancreatol, 14: 237-244, 1993
131. *Takeda Y, Escribano MJ:* Effects of insulin and somatostatin on the growth and the colony formation of two human pancreatic cancer cell lines. J Cancer Res Clin Oncol 117: 416-20, 1991
132. *Tang C, Biemond I, Verspaget HW, Offerhaus GJ, et al:* Expression of somatostatin receptors in human pancreatic tumor. Pancreas 17: 80-84, 1998
133. *Tezel E, Nagasaka T, Tezel G, et al:* Characterization of scattered neuroendocrine cells in ductal carcinoma of the pancreas. Pancreas 25: 136-141, 2002
134. *Tomao S, Romiti A, Massidda B, et al:* A phase II study of gemcitabine and tamoxifen in advanced pancreatic cancer. Anticancer Res 22: 2361-2364, 2002
135. *Upp JR, Olson D, Poston GJ, et al:* Inhibition of growth of two human pancreatic adenocarcinomas in vivo by somatostatin analog SMS 201-995. Am J Surg 155: 29-35, 1988
136. *Van Laethem JL, Bourgeois V, Parma J, et al:* Relative contribution of Ki-ras gene analysis and brush cytology during ERCP for the diagnosis of biliary and pancreatic diseases. Gastrointest Endosc 47: 479-485, 1998
137. *Vernejoul F, Faure P, Benali N, et al:* Antitumor effect of *in vivo* somatostatin receptor subtype 2 gene transfer in primary and metastatic pancreatic cancer models. Cancer Res 62: 6124-6131, 2002
138. *Wakasugi H, Funakoshi A, Iguchi H, et al:* Pancreatic carcinoma associated with chronic pancreatitis. Intern Med 38: 951-956, 1999
139. *Wang M, Abbruzzese JL, Friess H, et al:* DNA adducts in human pancreatic tissues and their potential role in carcinogenesis. Cancer Res 58: 38-41, 1998
140. *Wang R, Li J, Rosenberg L:* Factors mediating transdifferentiation of islets of Langerhans to duct epithelial-like structures. J Endocrinol 171: 309-318, 2001
141. *Weiderpass E, Vainio H, Kauppinen T, et al:* Occupational exposures and gastrointestinal cancers among Finnish women. J Occup Environ Med 45: 305-15, 2003
142. *Welch HG, Schwartz LM, Woloshin S:* Are increasing 5-year survival rates evidence of success against cancer? JAMA 283: 2975-2978, 2000
143. *Wenger FA, Jacobi CA, Siderow A, et al:* Hormontherapie des postoperative rezidivierten Pankreaskarzinoms mit Octreotid und Tamoxifen. Chirurg 70: 694-699, 1999
144. *Wenger FA, Kilian M, Mautsch I, et al:* Influence of octreotide and tamoxifen in tumor growth and liver metastasis in N-nitrosobis(2-oxopropyl)amine induced pancreatic cancer in Syrian hamsters. Horm Res 54: 74-77, 2000
145. *Woutersen RA, Appel MJ, Van Garderen-Hoetmer A:* Modulation of pancreatic carcinogenesis by antioxidants. Food Chem Toxicol 37: 981-984, 1999
146. *Yao GY, Zhou JL, Lai MD, et al:* Neuroendocrine markers in adenocarcinomas: an investigation of 356 cases. World J Gastroenterol 9: 858-861, 2003
147. *Yip-Schneider MT, Sweeney CJ, Jung SH, et al:* Cell cycle effects of nonsteroidal anti-inflammatory drugs and enhanced growth inhibition in combination with gemcitabine in pancreatic carcinoma cells. J Pharmacol Exp Ther 298: 976-985, 2001
148. *Zalatnai A, Pogány V:* Apoptosis-induction and phosphorylation state in human pancreatic carcinoma xenografts following octreotide treatment. Anticancer Res 21: 477-480, 2001
149. *Zalatnai A, Schally AV:* Responsiveness of the hamster pancreatic cancer to the treatment with microcapsules of D-Trp-6-LH-RH and somatostatin analog RC-160: histological evidence of improvement. Int J Pancreatol 4: 149-160, 1989
150. *Zalatnai A, Schally AV:* Treatment of the N-Nitrosobis(2-oxopropyl)amine-induced pancreatic cancer in Syrian golden hamsters with D-Trp-6-LH-RH and somatostatin analog RC-160 microcapsules. Cancer Res 49: 1810-1815, 1989
151. *Zalatnai A, Szegedi Z, Bocsi J:* Flow cytometric evidence of apoptosis in human pancreatic cancer xenografts treated with Sandostatin (octreotide). Anticancer Res 20: 1663-1666, 2000
152. *Zalatnai A, Timár F:* In vitro antiangiogenic effect of Sandostatin (octreotide) on the proliferation of the placental vessels. Anticancer Res 22: 4225-4228, 2002
153. *Zugmaier G, Jager R, Grage B, Gottardis MM, Havemann K et al:* Growth-inhibitory effects of vitamin D analogues and retinoids on human pancreatic cancer cells. Br J Cancer 73: 1341-1346, 1996