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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

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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)

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 selec-

ted topics of the pancreatic cancer: major problems, intriguing facts, controversies and some promising approaches.

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

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are highly variable (ranging from 6% wise 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.

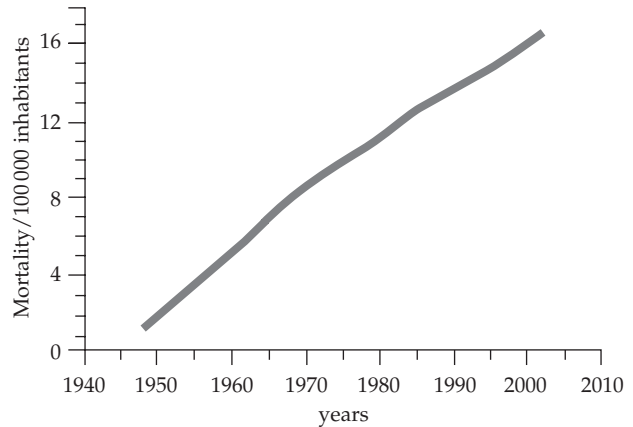


Figure 1. Mortality trends of pancreatic cancer in Hungary between 1948 and 2002 displaying a 15-fold increase in incidence.

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 TSNA.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 manufacturing²⁵ 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, they 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.

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 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 molel-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 its 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 phenomena 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/pre-neoplastic 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

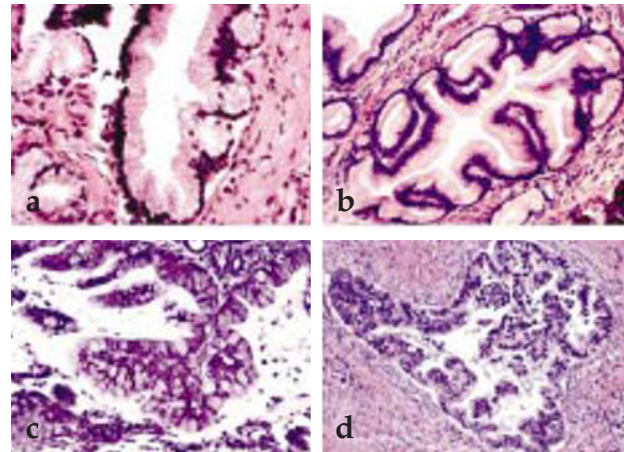


Figure 2. Histological spectrum of the pancreatic intraepithelial neoplasia (PanIN). (a) PanIN-1A: flat mucinous hyperplasia in the small ducts (HE, x200); (b) = PanIN-1B: papillary mucinous hyperplasia without any nuclear changes (HE, x100); (c) = PanIN-2: in the papillary proliferation the nuclei are 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).

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 in 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.

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-neoplastic 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

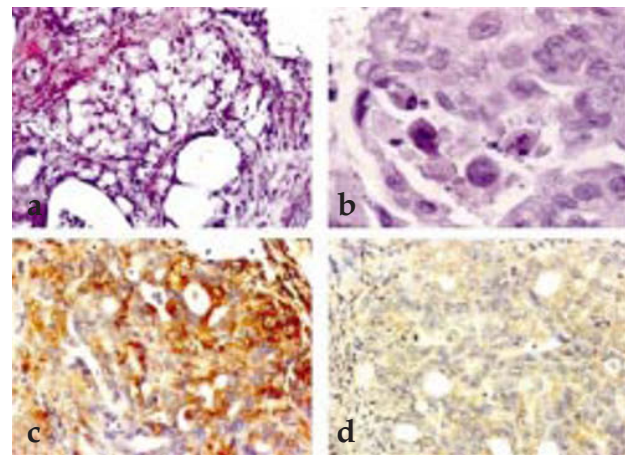


Figure 3. Hormonal sensitivity of the pancreatic carcinoma. (a) Apoptotic cells in nitrosamine-induced hamster pancreatic cancer after treatment with somatostatin analog (HE, x100); (b) apoptotic activity in a human pancreatic adenocarcinoma xenograft (PZX-15/F4) after a 1-month treatment with octreotide (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-operated¹¹⁸ 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 TNF α 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) lin-

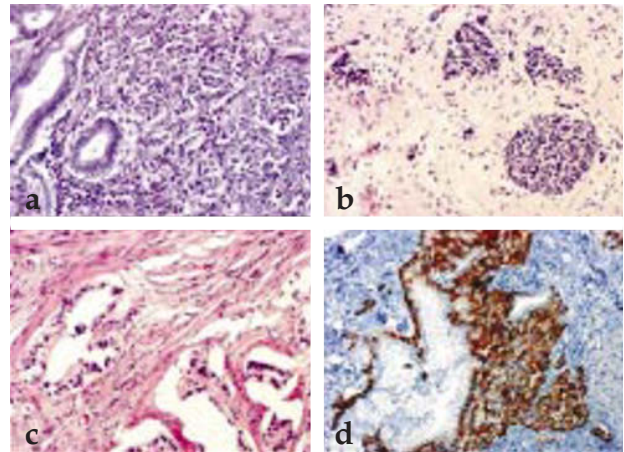


Figure 4. Mixed ductal-endocrine carcinoma. (a) intermingled exocrine and endocrine elements in the same tumor (HE, x100); (b) one part of the tumor is composed of clusters of proliferative endocrine cells (HE, x100); (c) another part of the same tumor showing an infiltrative adenocarcinoma component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

eages.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?

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 “retrodifferentiate” 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 et 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 proliferation⁸⁷ and the inhibitory effect was found to be correlated with the degree of immunohistochemical expression⁷². COX-2 inhibitors were also reported to potentiate the antiproliferative effect of gemcitabine.¹⁴⁷ However, some nonsteroidal anti-inflammatory drugs (NSAIDs) may be antimetabolic 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 breakthrough 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.

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