

Etiology and Oncogenesis of Pancreatic Carcinoma

Renata Dobrila-Dintinjana¹, Nenad Vanis², Marijan Dintinjana³ and Mladen Radić⁴

¹ University of Rijeka, Rijeka University Hospital Center, Department of Radiotherapy and Oncology, Rijeka, Croatia

² University Clinical Center, Division of Gastroenterology, Sarajevo, BiH

³ General medicine practice »Dr. Marijan Dintinjan Office«, Rijeka, Croatia

⁴ University of Rijeka, Rijeka University Hospital Center, Division of Gastroenterology, Department of Internal Medicine, Rijeka, Croatia

ABSTRACT

Pancreatic cancer is the fourth leading cause of cancer death overall¹. The factors that favor the development of pancreatic cancer can be divided into hereditary and acquired. Cancerogenesis is best explained by a »multi-hit« hypothesis, characterized with the developmental sequence of cellular mutations, forcing mutant cell to inappropriate proliferation and preventing its repair and programmed cell death (apoptosis). The most common mutations involve K-ras gene, epidermal growth factor (EGF-R) and HER2 gene. Continuous stimulation and secretion of vascular endothelial growth factor (VEGF) enhances the permeability of blood vessels provides nutrient supply to tumor site through newly formed vascular channels. This phenomena is known as vasculogenic mimicry. Loss of function of tumor-suppressor genes has been documented in pancreatic cancer, especially in CDKN2a, p53, DPC4 and BRCA2 genes. SDKN2A gene inactivation occurs in 95% of pancreatic adenocarcinoma. As regards acquired factors, smoking is only confirmed risk factor that increases the risk of pancreatic cancer. Diabetes, alcohol consumption, central obesity in men, infection with Helicobacter pylori and chronic pancreatitis are suspected, but not proven risk factors. Consumption of fruits and vegetables does not protect, while the consumption of meat processed at high temperatures increases the risk of pancreatic cancer. According to some studies, lycopene and folate levels are reduced in pancreatic carcinoma patients, reduced folate intake increases the risk of pancreatic carcinoma (48%), and this risk can be diminished by introducing folate-rich foods to diet, not by using pharmaceutical products. Occupational exposure to chlorinated hydrocarbons, vinyl chloride, nickel, chromium, insecticides and acrylic amide minimally increases the risk for pancreatic cancer. Exposure to cadmium (metal industry) associated with smoking result in the accumulation of cadmium in pancreatic tissue and the possible impact on carcinogenesis.

Key words: pancreas, pancreatic carcinoma, etiology, oncogenesis, hereditary neoplastic syndromes, hereditary factors

Introduction

It is estimated that 200–220 000 people worldwide die from pancreatic cancer every year. Moreover, according to autopsy data, about 30% of all cancers of unknown primary site are in fact carcinoma of the pancreas; therefore, these 50–80 000 cases should also be taken into consideration and added to. Pancreatic carcinoma, accounting for 3% of all malignant tumors, is responsible for 5% of all cancer deaths; pancreatic cancer is the fourth leading cause of cancer death overall¹.

Data from the National Cancer Institute (NCI) show that in the United States, 38 000 people are diagnosed with pancreatic cancer and about 34 300 (90%) people die from the disease each year. The incidence rate is 13 cases

per 100 000 men and women per year, showing an annual steady pattern in the population in the last 10 years¹. The largest number of cases occur between 70 and 75 years of age, with a linear increase starting at age 45 and only 2% of patients under age 40. Data for Croatia and Europe are similar to those listed above. Compared to Europe, North America and Japan, the incidence rate of pancreatic carcinoma reported for African and Asian countries is significantly lower (1–6/100 000). Furthermore, African Americans have a significantly higher incidence rate than Caucasian Americans (11.4 f and 16.6 m vs 7.4 f and 11.4 m)². Migration studies have shown that the risk of developing pancreatic cancer in migrants cor-

responds to the incidence rate in their new place of residence in two successive generations. This shows that environmental factors play a role in the development of pancreatic cancer². Only 7% of pancreatic cancer cases are detected at the stage when the tumor is confined to the pancreas. The five-year survival for this stage is 20%. In the majority of patients, however, the tumor is detected only as metastatic disease (52%), with the five-year survival rate of 1.8%³. The five-year overall survival rate is less than 5%, with less than one-quarter of patients (24%) surviving their first year after diagnosis³. Advances in medicine and the improvement of diagnostic techniques have not had a significant impact on reducing morbidity and mortality from this disease in the last 10 years. This fact becomes even clearer in the context of knowing that pancreatic carcinoma is a tumor in which the vasculogenic mimicry phenomenon occurs at the earliest stage of malignant cell proliferation⁴.

Neuroendocrine carcinomas, mucinous cystadenocarcinomas or intraductal papillary-mucinous carcinomas have a better prognosis as regards five-year survival.

Etiology and Oncogenesis

Despite intensive research, the etiology of pancreatic cancer remains largely unknown. However, oncology studies have provided important information on risk factors for developing the disease. The factors that favor the development of pancreatic cancer can be divided into hereditary and acquired⁵.

The hypothesis that for the time being, best explains the process of pancreatic carcinogenesis is a »multi hit« hypothesis of multiple »hits«, i.e. the chronological sequence of development and a series of multiple mutation events within a single cell, which encourage the cell to divide inappropriately and prevent both its repair and programmed cell-death (apoptosis)^{6,7}. The sequence begins with the mutation of genes whose products are within the system of receptors in which signal transduction in the cell is of the utmost importance for cell-cycle regulation, proliferation and differentiation. The mutation of these genes creates oncogenes whose products are altered molecules which also alter receptor activities, usually by affecting the increased or continuous activity of such receptors. Oncogenes can be inherited or produced by a de novo mutation. Some external factors such as a diet rich in saturated fats, smoking or a permanent state of inflammation of the pancreas can lead to increased synthesis of oxygen radicals and affect gene mutations.

Oncogene mutation is the first »hit« in a series of mutations occurring in a cell. Such cell still retains mechanisms capable of suppressing the oncogene effects or, on the other hand, maintaining the capacity to repair DNA damage, and inducing mechanisms of apoptosis – programmed cell death in case of cell damage. Protein products regulating the above processes are encoded by a group of genes called tumor-suppressor genes. The second »hit« represents a mutation of these very genes. The

mutated gene can be inherited or there is a possibility that mutations are acquired and arisen de novo.

Mutations of the K-ras gene occur in over 90% of pancreatic adenocarcinoma, and result in mutation of signaling G-protein. The mutated product ensures the continuous transmission of signals in the cell. On the other hand, enhanced autocrine signaling through the epidermal growth factor (EGF-R) and HER2 receptor plays a proven role in carcinogenesis. The induction of these proto-oncogenes and their products result in tumor growth, and angiogenesis. Continuous activation of Stat3 induces the transcription of anti-apoptotic regulatory protein Bcl and the expression of VEGF-r. The increased expression of growth factors (vascular endothelial growth factor – VEGF, fibroblast growth factors – FGFs, EGF), via an autocrine and/or paracrine mechanisms, stimulates growth of pancreatic cancer cells, which are then no longer dependent on growth stimulation from surrounding tissues⁸. Mutations in Smad4 and the overexpression of Smad6 and 7 lead to pancreatic cancer cell resistance to inhibitory effects exerted on growth by the transforming growth factor- β (TGF- β) family⁸.

Pancreatic cancer cells have successfully developed a series of mechanisms that allow the invasion of surrounding tissues and the development of distant metastases. This is especially assisted by the hepatocyte growth factor (HGF), nerve growth factor (NGF) and TGF- β , as shown in Table 1.

While oxygen saturation (pO₂) levels in various tissue range between 24–6 mmHg, oxygenation levels in tumor tissues are much lower (10–30 mmHg), and pancreatic cancer tissue shows a saturation level of only 3 mm Hg. Although there are no clinical studies confirming the relationship between extreme hypoxia and the absence of response to chemotherapy and radiation therapy, it is assumed that conditions of extreme hypoxia have adversely affect treatment results⁹. Moreover, hypoxia is the strongest stimulator of the secretion of VEGF and without VEGF there would be no tumor vascularization and progression of the disease. VEGF activates mitogen signaling pathways, induces vascular permeability and improves permeability of blood vessels around the tumor mass. Thus, nutrients can reach tumor cells via vascular channels formed by these tumor cells, a phenomenon known as vasculogenic mimicry⁹.

The loss of function of some tumor-suppressor genes has been documented in pancreatic carcinoma, especially of CDKN2a, p53, DPC4 and BRCA2. Inactivation of the SDKN2A gene occurs in 95% of pancreatic adenocarcinomas. This mutation determines refractoriness of the tumor to irradiation. In 50–60% of adenocarcinoma cases the p53 mutation occurs, usually during transition from a lower to higher stage malignancy. Damage in the p53 gene is associated with cell cycle disorder, transcription, DNA repairs and apoptosis. Inactivation of the DPC4 gene occurs in 55% of pancreatic carcinoma, and leads to the inability to maintain adequate regulation of TGF- β expression and subsequent inability to inhibit cell growth. Mutations of the BRCA2 gene are also known to occur in

10% of pancreatic cancer as well as STK11 gene mutations characteristic for Peutz-Jeghers syndrome. Mutations of genes whose products are involved in DNA base repair, such as MLH1 and MSH2, have been shown in less than 5% of pancreatic cancer cases.

Almost 11% of families with *brca1-brc2* mutation have at least one relative suffering from cancer of the pancreas¹⁰. Five to 10% of patients have a genetic predisposition to develop the disease. In such patients, the disease develops at an earlier age, than in with sporadic cancer. There are hereditary syndromes that are associated with an increased risk of developing pancreatic carcinoma. This includes families with frequent incidence of pancreatic cancer, families with increased incidence of all cancers, hereditary syndromes associated with an increased risk of pancreatic cancer and genetic predisposition for other diseases that at the same time increase the risk to develop pancreatic cancer. Hereditary syndromes, gene mutations, and the risk of developing pancreatic cancer are shown in Table 1.

Comparing the results obtained in cytogenetic studies with pathohistological findings it has been shown that 60–70% of patients have an abnormal karyotype; 40% of them have one numerical or structural abnormality, and 60% have complex multi-chromosomal changes¹⁰. Patients with complex changes more often have poorly differentiated cancers with poorer prognosis. Karyotype is an independent prognostic factor. Although the pattern of autosomal dominant inheritance (pancreatic cancer-specific PNCA1-locus on chromosome 4q32-34), the specific gene remains unidentified¹⁰.

Hereditary pancreatitis is an autosomal dominant disease with high penetration and variable expression, and the development of chronic pancreatitis in the first or second decade of life. These patients have a 50 time

greater risk of developing pancreatic cancer in their fifth decade of life, and among them smokers are especially at risk. The risk is also increased in patients with cystic fibrosis¹¹.

As regards acquired factors, it has been proven that smoking increases the risk of pancreatic cancer by 1.5 to 5 times¹². The risk is reduced by smoking cessation, and becomes level with the risk in non-smokers after 10–15 years of smoking abstinence. Tobacco is a component that affects late stages of carcinogenesis. About 60-odd carcinogenes have been found in cigarettes; nicotine-derived nitrosamine ketons bind to DNA and induce mutations in the K-ras gene. While both nicotine chewing and nicotine inhaling is equally carcinogenic as cigarette smoke, there are no conclusive studies showing the noxious effects of both cigar and pipe smoking¹³.

Patients suffering from diabetes for more than 5 years have a 2 time higher risk of pancreatic cancer compared to a nondiabetic population, as concluded in a large meta-analysis derived from 20 studies¹⁴. The question that arises out of other studies is whether diabetes is the cause or the consequence of pancreatic cancer, particularly in elderly, nonobese patients without family history of diabetes. People suffering from atopic allergies and patients receiving metformin for their diabetes are at a lower risk of developing pancreatic cancer.

A large number of studies have shown that central obesity is related with the increased incidence of pancreatic cancer in men, whereas there is no such evidence for women. Central adiposity is associated with increased insulin secretion and insulin resistance as well as increase in intra-abdominal fat¹⁵.

While viruses do not play a significant role in the etiology of pancreatic cancer, infection with *Helicobacter pylori* increases the risk of developing pancreatic cancer

TABLE 1
SYNDROMES ASSOCIATED WITH AN INCREASED RISK OF PANCREATIC ADENOCARCINOMA

Syndrome	Gene(s)	Relative Risk	Total Risk	Chromosome
Exocrine Carcinoma				
Negative family history	–	1	0.5%	–
Hereditary breast/ovarian cancer	BRCA2	3.5–10x	5%	13q
Dysplastic nevus syndrome (FAMM)	CDKN2A	20–34X	17%	9p
>2 familial pancreatic cancer among first-degree relatives	–	32x	16%	–
Hereditary chronic pancreatitis	PRSS1, SPINK1	50–80x	40%	7q,5q
Peutz-Jeghers syndrome	STK 11	75–130x	36%	19p
Familial adenomatous polyposis	APC	Unknown	Unknown	5q
Hereditary nonpolyposis colorectal cancer syndrome (Lynch II)	DNA mismatch repair gene (LCF2)	Unknown	<5%	2p, 3p, 7p
Ataxia telangiectasia	ATM	Unknown	Unknown	11q
Li-Fraumeni syndrome	p53	Unknown	Unknown	17p
Endocrine Carcinoma				
MEN-1	MEN 1(MENIN)	Unknown	50%	11
Von Hippel-Lindau	VHL	Unknown	17%	3p

by 2 times; *Helicobacter pylori* stimulates gastrin and reduces somatostatin secretion which results in stimulation of pancreatic cancer cell growth and promotes carcinogenesis. In addition, there is also increased acidity, increased DNA synthesis, increased formation of N-nitroso components and chronic inflammation, which is also an introduction to carcinogenesis¹⁶.

A meta-analysis combining the results of 17 studies has not confirmed that alcohol consumption increases the risk of pancreatic cancer, unless chronic pancreatitis has developed due to alcohol abuse¹⁷. Acquired long-term chronic pancreatitis increases the risk of developing pancreatic cancer by 26 times, or 4% of patients suffering from chronic pancreatitis for 20 years will develop cancer of the pancreas¹⁸.

Although some studies have revealed the risk of developing pancreatic cancer to be lower in people consuming plenty of fruits and vegetables, it will be reasonable to reconsider their interpretation; people who consume fruits and vegetables regularly smoke less. Therefore, there is no surprise in conclusions reached in a study that »head to head« opposed the so-called »prudent diet« *vs.* »western diet« and proved the same risk of pancreatic cancer in both groups. Consumption of meat processed at high temperature (heterocyclic amines and N-nitroso components) seems to increase the risk of developing pancreatic cancer¹⁷. As proven *in vitro*, the green tea antioxidants have a beneficial effect on reducing the risk of pancreatic cancer, although no such evidence has been provided *in vivo*. Also, there is no evidence whatsoever that coffee consumption may raise the cancer risk¹⁹.

REFERENCES

1. MAISONNEUVE P, LOWENFELS AB, *Dig Disease*, 28 (2010) 234. DOI:10.1159/000320068. — 2. THOMAS DB, KARAGAS MR, *Cancer Res*, 47 (1987) 5771. — 3. RIES LAG, MELBERT D, KRAPCHO M, STINCHCOMB DG, HOWLADER N, HORNER MJ, National Cancer Institute, Bethesda, MD. — 4. HENDRIX MJC, SEFTOR EA, *Nature Reviews Cancer*, 3 (2003) 411. DOI: 10.1038/nrc1092. — 5. TERSMETTE AC, PETERSEN GM, OFFERHAUS GJ, *Clin Cancer Res*, 3 (2001) 738. — 6. SCHNEIDER G, SCHMID RM, *Mol Cancer*, 2 (2003) 15. DOI: 10.1186/1476-4598-2-15. — 7. HAMACHER R, SCHMID RM, SAUR D, SCHNEIDER G, *Mol Cancer*, 7 (2008) 64. DOI: 10.1186/1476-4598-7-64. — 8. BLACKFORD A, SERRANO OK, WOLFGANG CL, PARMIGIANI G, JONES S, ZHANG X, PARSONS DW, *Clin Cancer Res*, 15 (2009) 4674. DOI: 10.1158/1078-0432.CCR-09-0227. — 9. BÜCHLER P, REBER HA, BÜCHLER MW, FRIESS H, HINES OJ, *Ann Surg*, 236 (2002) 738. DOI: 10.1097/0000658-200212000-00006. — 10. PETERSEN GM, AMUNDADOTTIR L, FUCHS CS, KRAFT P, STOLZENBERG-SOLOMON RZ, JACOBS KB, *Nature Genet*, 42 (2010) 224. DOI: 10.1038/ng.522. — 11. MAISONNEUVE P, MARSHALL BC, LOWENFELS AB, *Gut*, 56 (2007) 1327. DOI: 10.

In pancreatic cancer patients the level of antioxidants is much below the limits of normal. Hence, high hopes have been placed in various nutritional supplements (alpha-tocopherol, ascorbic acid, zinc, selenium), however, for the time being, there is no conclusive evidence on their beneficial effect²⁰. According to some studies, lycopene and folate levels are reduced in pancreatic carcinoma patients, reduced folate intake increases the risk of pancreatic carcinoma (48%), and this risk can be diminished by introducing folate-rich foods to diet, not by using pharmaceutical products²¹.

Occupational exposure to chlorinated hydrocarbons, vinyl chloride, nickel, chromium, insecticides and acrylamide minimally increases the risk of developing pancreatic cancer. Exposure to cadmium (metal industry) combined with smoking result in the accumulation of cadmium in pancreatic tissue and potential impact on carcinogenesis²².

We may conclude that the knowledge of the etiology of pancreatic cancer has significantly improved in the last decade. A gradual change from normal pancreatic tissue to fully malignant one has been shown; progressive alterations occurring at both the genetic and tissue level are now known. Although there are several hereditary factors associated with pancreatic cancer, the hereditary disease still accounts for only 10% of all pancreatic carcinoma cases. Avoiding smoking and dietary modifications are the only option now that may reduce the risk of the disease. It may be expected that new molecular and imaging techniques will enable clinicians to detect pancreatic cancer at an early, potentially curable stage.

1136/gut.2007.125278 — 12. MACK TM, YU MC, HANISCH R, HENDERSON BE, *J Natl Cancer Inst*, 76 (1986) 49. — 13. SHAPIRO JA, JACOBS EJ, THUN MJ, *J Natl Cancer Inst*, 92 (2000) 333. DOI: 10.1093/jnci/92.4.333. — 14. STEVENS RJ, RODDAM AW, BERAL V, *Br J Cancer*, 96 (2007) 507. DOI: 10.1038/sj.bjc.6603571. — 15. CALLE EE, RODRIGUEZ C, WALKER-THURMOND K, THUN MJ, *N Engl J Med*, 348 (2003) 1625. DOI:10.1056/NEJMoa021423. — 16. RISCH HA, *J Natl Cancer Inst*, 95 (2003) 948. DOI:10.1093/jnci/95.13.948. — 17. LOWENFELS AB, MAISONNEUVE P, CAVALLINI G, AMMANN RW, LANKISCH PG, *Int J Cancer*, 328 (1993) 1433. DOI: 10.1002/ijc.20800. — 18. LOWENFELS AB, MAISONNEUVE P, CAVALLINI G, *N Engl J Med*, (1993) 328. DOI:10.1056/NEJM199305203282001. — 19. TAVANI A, LA VECCHIA C, *Eur J Cancer Prev*, 9 (2000) 241. — 20. ZULLO A, FERREIRA PR, ERTEKIN MV, ZHAN CD (2000-2004) — 21. LARSSON SC, HÅKANSSON N, GIOVANNUCCI E, WOLK A. *J Natl Cancer Institute*, 98 (2006) 407. DOI: 10.1093/jnci/djj094. — 22. OJAJÄRVI A, PARTANEN T, AHLBOM A, BOFFETTA P, HAKULINEN T, *Am J Epidemiol*, 153 (2001) 841. DOI: 10.1093/aje/153.9.841.

R. Dobrila-Dintinjana

University of Rijeka, Rijeka University Hospital Center, Clinic for Radiotherapy and Oncology, Krišimirova 42, Rijeka, Croatia

e-mail: renatadobrila@windowslive.com

ETIOLOGIJA I ONKOGENEZA KARCINOMA GUŠTERAČE

S A Ž E T A K

Karcinom gušterače je četvrti vodeći uzrok smrti od karcinoma¹. Čimbenici koji pogoduju pojavi karcinoma gušterače dijele se na nasljedne i stečene. Hipoteza koja za sada najbolje objašnjava proces kancerogeneze u stanicama pankreasa je »multi hit« hipoteza višestrukog »udarca«, tj. kronološkog slijeda razvoja i zadržavanja višestrukih mutacija unutar jedne stanice, a koji ju tjeraju u nesvrsishodnu diobu i onemogućavaju njenu reparaciju i programiranu smrt (apoptozu). Slijed započinje mutacijom gena i nastankom onkogeno čiji produkti mijenjaju stanje aktivnosti receptora. Produkti – proteini koji reguliraju navedene procese kodirani su u skupini gena koje nazivamo tumor-supresorskim genima. Drugi »udarac« predstavlja mutaciju tumor supresorskih gena, koji imaju funkciju prigušenja učinaka onkogeno popravak oštećene DNA ili inicijaciju apoptoze – programirane smrti stanice. Najčešće mutacije zahvaćaju K-ras gen, epidermalni čimbenik rasta (EGF-R) i HER2 gen. Važnu ulogu igra i trajna stimulacija sekrecije VEGF-a, hipoksijom u tumorskom tkivu, bez kojega nema vaskularizacije tumora i progresije bolesti. VEGF poboljšava propusnost krvnih žila u okolini tumorske mase. Nutritijenti potom mogu doći do tumorskih stanica kroz vaskularne kanale koje formiraju same stanice, koji fenomen se naziva vaskulogena mimikrija. Gubitak funkcije tumor-supresorskih gena dokumentirana je u karcinomu gušterače, posebice CDKN2a, p53, DPC4 i BRCA2. Inaktivacija SDKN2A gena javlja se u 95% adenokarcinoma gušterače. Od stečenih čimbenika pušenje je jedini dokazani čimbenik koji povećava rizik od karcinoma gušterače. Šećerna bolest, konzumacija alkohola, centralni tip debljine u muškaraca, infekcija s *Helicobacter pylori*, kronični pankreatitis se spominju kao mogući čimbenici, ali nisu potvrđeni kao sigurni čimbenici rizika za karcinom gušterače. Konzumacija voća i povrća ne štiti, dok konzumacija mesa obrađenog na visokim temperaturama povećava rizik od nastanka karcinoma gušterače. Za sada nema konkluzivnih dokaza o učinku antioksidansa (alfa-tokoferol, askorbinska kiselina, cink, selenijum) na karcinom pankreasa iako smanjen unos folata podiže rizik za nastanak karcinoma gušterače, a rizik se može smanjiti dodatkom folata iz hrane. Profesionalna izloženost klorinat hidrokarbonu, vinil kloridu, niklu, kromijumu, insekticidima i akril amidu minimalno povisuje rizik za karcinom gušterače. Izloženost kadmiju (metalna industrija) udruženo s pušenjem dovodi do akumulacije kadmija u pankreatičnom tkivu i mogućeg utjecaja na karcinogenezu.