ASSOCIATED RISK FACTORS FOR PANCREATIC CANCER: POTENTIAL TARGETS IN EARLY DETECTION STRATEGIES

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ASSOCIATED RISK FACTORS FOR PANCREATIC CANCER: POTENTIAL TARGETS IN EARLY DETECTION STRATEGIES (Abstract) : The common risk factors for pancreatic cancer (PC) are smoking, high alcohol consumption, obesity, family history of PC and dietary factors. Chronic pancreatitis facilitates the progression to cancer, resulting in the occurrence of precancerous lesions. Genetic factors variation or mutation contribute to increase risk of PC. The biomarker combination CA19-9 (Carbohydrate antigen 19-9, also known as sialyl-Lewis) along with MUC5AC (Mucin 5AC - this gene has been linked to mucus hypersecretion) is also suggestive for PC diagnosis. Transabdominal ultrasound, contrast enhanced computed tomography or magnetic resonance imaging are used to detect PC, but positron emission tomography with fluoro-D-glucose is clearly superior in early detection of small tumors. Keywords: PANCREATIC CANCER, RISK FACTORS, EARLY DETECTION.

Pancreatic cancer (PC) is an aggressive and invariably deadly disease without any improvements in patient outcome over the last 2 decades. The estimated number of deaths in 2013 was around 39,000, making PC the fourth leading cause of cancer deaths in the USA, with over 45,220 new cases diagnosed every year (1).

Epidemiology
The worldwide incidence of pancreatic ductal adenocarcinoma (PDAC) is 6-12/100,000/year (2) and it is seldom diagnosed before 55 years old. PDAC can be defined as a disease of elderly populations with the highest incidence in people older than 70 (3).The incidence of PDAC varies across regions and populations, possibly because of the environment and/or the exposure to certain risk factors.

Risk factors for PC
The etiology of PC represents the subject of numerous meta-analyses and pooled analyses in order to establish several risk factors. The most common risk factor is
tobacco smoking, followed by obesity, high alcohol consumption, history of pancreatitis and diabetes, family history of PC and possibly selected dietary factors (4).

Acute pancreatitis (AP), a sterile inflammation initiates from acinar cell injury following a series of impairment of pancreatic enzymes activation processes, increased inflammatory responses and ultimately cell death with the complication of multiple organ failure (5, 6). The most common causes of pancreatitis include alcohol, gallstones, toxins, hyperlipidemia, and trauma, with a small number of idiopathic cases. Traditionally AP is self-limited with complete resolution of function after the acute event, but sometimes there may be stricture formation, leading to pancreatic flow obstruction and recurrent AP (RAP). Recurrent episode of AP would progress to chronic pancreatitis (CP), which is a well-defined risk factor for PC (1). There are three stages of CP development: stage one (the pre-pancreatitis phase), associated with risk factors for this transition, like alcohol consumption and tobacco smoking (6). Then, stage two (AP form) with release of inflammatory cytokines which, in the severe forms, it could activate macrophage dependent stellate cells, which finally could lead to fibrosis. The third stage is represented by the progression to CP driven by the factors that modulate immune responses (7).

In one recent study of 731 prospectively enrolled patients after a primary episode of AP, the risk of future development of PDAC was 0.7%, after median of 55 months of follow-up. The study also showed that the risk of PC was 9 times higher in patients who developed CP (8). Another study among 790 patients with histologically confirmed PC, 114 (14.4%) had a history of pancreatitis (AP within 2 years of PC diagnosis in 69 [8.7%], remote history of AP in 28[3.5%], CP in 4[0.5%], and unknown duration of pancreatitis in 13[1.6%]. After controlling for sex, age, body mass index, smoking, alcohol history, and diabetic status at diagnosis, patients with a remote history of AP were diagnosed on average 4.7 years earlier with PC compared with PC patients without history of AP (9).

CP is a refractory disorder characteristic of progressive inflammation and fibrosis with irreversible damage to both exocrine and endocrine function of the pancreas. Acinar cell atrophy, pancreatic fibrosis, leukocytes infiltration, fatty replacement and blocked ducts represent the typical histopathologic features of CP (10). Both CP and PDAC include massive immune cell infiltration and intensive fibrosis (1).

A meta-analysis among patients with CP has shown a relative risk of 13.3% for developing PC (2). Chronic inflammation associated with CP facilitates the progression to cancer, resulting in the occurrence of three types of precancerous lesions: pancreatic intraepithelial neoplasia (PanINs), intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN). Subsequent evolution of these precursor lesions into PDAC ultimately involves a number of various molecular changes (11). Despite the strong link between CP and PC, less than 5% of patients with CP go on to develop the disease (2).

Among the non-modifiable risk factors are diabetes mellitus, age, genetic factors and gender. PC is more common in males than in females, possibly due to lifestyle (higher alcohol and tobacco consumption) (12).
Genetic factors contribute to increase risk of PC and the most important are genetic variation or mutation of BRCA1 (Breast cancer type 1 susceptibility protein), BRCA2, PALB2 (Partner and localizer of BRCA2, also known as PALB2), ATM (ataxia telangiectasia mutated), CDKN2A (cyclin-dependent kinase Inhibitor 2A), APC (Adenomatous polyposis coli), MLH1 (MutL homolog 1, colon cancer, nonpolyposis type 2 E. coli), MSH2 (DNA mismatch repair protein Msh2), MSH6, PMS2 (Mismatch repair endonuclease), PRSS1 (Mutations of Human Cationic Trypsinogen) and STK11 (Serine/threonine kinase 11). 5-17% of families with familial PC have been identified with Germ-line BRCA2 gene mutations, the highest proportion of known causes of inherited PC. Also, familial cancer syndromes (hereditary non-polyposis colon cancer, Peutz-Jeghers syndrome, hereditary breast and ovarian cancer syndrome, familial adenomatous polyposis and Li-Fraumeni syndrome) found to be associated with PC (13).

LABORATORY INVESTIGATIONS

Serum bilirubin levels are greater than 10 mg/dL in CP, with a peak followed by a fall as inflammation decreases (14). In PDAC, bilirubin progressively increases till biliary decompensation is done (15). When serum bilirubin >5.8 mg/dL, MPD> 11.5 mm, CBD>14.5 and CA 19-9 >127 UI/mL, the sensitivity for suspecting malignancy is 100% (16). Ca 19-9, the gold standard PDAC biomarker, is the single most important predictive biomarker of malignancy in mass forming CP with a sensitivity and specificity of 77% and 86% (17). However, the value can be elevated in obstructive jaundice, cholangitis, cirrhosis and CP in absence of malignancy (18). A proinflammatory marker of CXC chemokine family, interleukin 8, is found to be commonly expressed in PDAC (55.6%) and it can predict the prognosis (19).

Methylated biomarkers such as KRAS are found to be 95% specific and 75% sensitive for PDAC in comparison with normal controls and CP. A metabolic signature to distinguish PDAC from CP, with a diagnostic accuracy of 90%, contains CA 19-9 along with certain metabolites such as proline, 2 sphingomyelin, phosphatidylcholine, pyruvate, ceramide, histidine, sphingosine-1-phosphate and isocitrate, with an 85% sensitivity and 94,9% specificity (20).

The biomarker combination which is found to be very promising is CA 19-9 along with MUC5AC, a mucin family member produced by both well differentiated and poorly differentiated PDAC (17). MUC5AC is undetectable in normal pancreas, islet cell tumors and CP (21). Its levels are affected by elevated bilirubin levels. Serum levels can be measured by enzyme-linked immunosorbent assay, whereas tissue levels can be measured by immunohistochemistry (17).

A monoclonal antibody with high specificity for MUC1 (Mucin 1, cell surface associated) is PAM4, found to be expressed in PDAC as compared to normal pancreatic tissue and pancreatitis. It has a better sensitivity and specificity than CA 19-9 (22).

A recent study made by Lukasiewicz-Zajac et al. showed that matrix metalloproteinase-2 (MMP-2) and its tissue inhibitor (TIMP-2) are highly useful as potential biomarkers in the diagnosis of PC. MMP-2 is a proteolytic enzyme responsible for the degradation of the basal membrane and extracellular matrix. Imbalance between MMP-2 and TIMP-2 have a significant role
in invasion, migration, metastasis of tumoral cell and neoangiogenesis (23).

The study of Zhai et al. proved, by immunohistochemical methods, that the expression of MMP-2 was higher in PC tissue, fact that was positively correlated with higher CA 19-9 serum levels, with poor histological grade, advanced stage, perineural invasion, lymph node and distant metastasis (24).

Another study revealed a new combination of CA 19-9, thrombospondin-2 (THBS2) and circulating cell-free DNA (cfDNA) as a composite liquid biomarker for the diagnosis of early-stage PDAC. This panel showed a 92% sensitivity in discriminating PDAC from CP or IPMN (25).

**IMAGING**

Abdominal X-ray, transabdominal ultrasound (USG), contrast enhanced computed tomography (CECT) or magnetic resonance imaging (MRI) can be used to detect morphological changes in the pancreas, ductal findings, metastatic lesions, ascites, lymphadenopathy and vascular involvement (26, 27).

Radiologically, a malignancy in CP may appear like a lesion in diffuse CP or a mass forming focal CP (28).

USG can be useful especially when the patient has liver metastatic disease and/or ascites. In those cases, an USG-guided biopsy or fluid cytology can establish the diagnosis. A contrast-enhanced USG in arterial and venous phases is useful only when the malignancy is advanced with vascular involvement (29).

A triple-phase pancreas protocol CECT scan represents one of the main investigations. Frequently, PDAC is seen as a gradual, progressive hypo enhancing lesion with a delayed peak in the portal phase without washout and pancreatic parenchymal atrophy/PDAC, often localized in the head of pancreas, is associated with dilated CBD and MPD (double duct sign), with an abrupt cutoff at the mass. Vascular sheathing at origin of celiac artery and superior mesenteric artery by a hypodense mass is suggestive for malignancy (26). However, 23% of small PC (<20 mm) cannot be detected (30). Another causes of this low sensitivity may be represented by some pathological characteristics of the tumor such as lower tumor cellularity, less prominent tumor necrosis and more frequent intratumorally islet cells and acinar tissues (31).

MRI has a stronger contrast enhancement and a superior ability to detect PDAC when compared with CT (32). Diffusion-weighted MRI (DWI) can help detect pancreatic neoplasms with extracellular fibrosis or extremely dense cellularity, but it cannot definitively characterize solid lesions as neoplastic or inflammatory (33).

Positron emission tomography with 2-deoxy - 2[fluorine-18] fluoro-D-glucose (FDG-PET) is superior to MRI and CT, with 89% sensitivity and 88% specificity, being able to detect PC < 20 mm. For that matter, it is used for cancer staging and revealing recurrent lesions (34).

Endoscopic ultrasonography (EUS) become the most sensitive examination for PDAC in patients with high-risk factors for PDAC or with manifestations suggesting PC (35).

The conventional B-mode EUS imaging was complemented by ultrasound contrast agents (UCAs), which allows the evaluation of vascular structures. Contrast-enhanced high mechanical index (CEHMI) EUS and contrast-enhanced low mechanical index (CELMI) are helpful for detecting
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macro vessels, respectively capillary vascularization patterns inside the area of interest. Endoscopic son elastography (ESE) tests the hardness of tissue and benign tissue is more pliant compared to a malignant tumor. A recent study made by Finn-Jorn et al. showed that CEHMI-EUS can detect PDAC and differentiate it from CP more effectively than CT, conventional EUS, ESE and CELMI-EUS (36).

ERCP has a low sensitivity for diagnosing PC, being considered to be inadequate for examination. Besides, post-ERCP pancreatitis is a very unpleasant adverse event (37). Serial pancreatic juice aspiration cytologic examination (SPACE) was recently developed. It proved to have a high sensitivity and specificity even for pancreatic carcinoma in situ or small lesions (38).

**CONCLUSIONS**

Pancreatic cancer is a major health problem, being one of the most aggressive forms of cancer.

Acute and chronic inflammation associated with CP facilitates the progression to cancer, resulting in the occurrence of pre-cancerous lesions.

CA 19-9 with MUC5AC combination may become an ideal investigation in PC detection strategies (39).

FDG-PET is superior to MRI and CT, with high sensitivity and specificity, being able to detect PC < 2 cm.

REFERENCES


Associated risk factors for pancreatic cancer: potential targets in early detection strategies


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**POLYMERIC NANOPARTICLES FOR ORAL DELIVERY OF INSULIN**

Biomacromolecules have transformed our capacity to effectively treat diseases; however, their rapid degradation and poor absorption in the gastrointestinal (GI) tract generally limit their administration to parenteral routes. An oral biologic delivery system must aid in both localization and permeation to achieve systemic drug uptake. Inspired by the leopard tortoise’s ability to passively reorient, we developed an ingestible self-orienting millimeter-scale applicator (SOMA) that autonomously positions itself to engage with GI tissue. Gastrointestinal responsive polymeric nanospheres (NPs) based on hydroxypropyl methylcellulose phthalate (HPMCP) were prepared using spontaneous emulsification solvent diffusion method for improved oral administration of insulin. The NPs were prepared under optimized conditions have an encapsulation efficiency of 90% and a particle size of about 200 nm. In vitro drug release experiments demonstrated that the NPs exhibited a gradient release profile of loaded drug when the pH value gradually increased from 3.0 to 7.4. Enzyme resistance experiments showed that under simulated gastrointestinal conditions, the NPs protected more than 60% of the drug from being degraded by trypsin. The oral hypoglycemic experiments revealed that insulin-loaded NPs could significantly reduce blood glucose levels in diabetic rats with a relative bioavailability of 8.6%. *Ex vivo* imaging investigation of rat tissues showed that the drug-loaded NPs could promote the absorption of insulin in the ileum and colon. The work described here suggests that the gastrointestinal responsive polymeric NPs may be promising candidates for improving gastrointestinal tract delivery of hydrophilic biomacromolecules. Accordingly, the results indicated that HPMCP NPs with gastrointestinal stimuli responsiveness could be a promising candidate for oral insulin delivery (Y. Fang, Q Wang, Lin X, *et al.* Gastrointestinal responsive polymeric nanoparticles for oral delivery of insulin: optimized preparation, characterization and *in vivo* evaluation. *J Pharm Sci* 2019; 108(9): 2994-3002).