Nutritional Links to Plausible Mechanisms Underlying Pancreatic Cancer

Executive Summary

Nutritional Sciences Research Group and

Cancer Biomarkers Research Group

Division of Cancer Prevention, National Cancer Institute

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I. INTRODUCTION

Recent epidemiological and experimental studies suggest that pancreatic cancer may be a form of cancer that is preventable. The NCI's 2002 Strategic Plan for Addressing the Recommendations of the Pancreatic Cancer Progress Review Group highlighted the need to evaluate environmental risk factors and gene-environment interactions and to identify biomarkers of early disease. Thus, it is timely to pose questions about how dietary factors and/or nutritional status affect the pathophysiology of cancer of the pancreas.

A workshop on "Nutritional Links to Plausible Mechanisms Underlying Pancreatic Cancer," cosponsored by the Division of Cancer Prevention's (DCP) Nutritional Science Research Group (NSRG) and Cancer Biomarkers Research Group (CBRG) was held on December 3, 2002, in Bethesda, Maryland. The workshop provided an opportunity for representatives from the extramural research community, intramural scientists from the National Cancer Institute (NCI), National Institute on Alcohol Abuse and Alcoholism (NIAAA), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and the National Center for Complimentary and Alternative Medicine (NCCAM) to discuss data and exchange ideas.

The NSRG's focus has been to identify nutrients that modify the cancer process, and to identify sites and mechanisms of action that are critical to the understanding of diet and cancer, including pancreatic cancer. In addition, the DCP's Early Detection Research Network (EDRN) is investigating the role of biomarkers, including biomarkers related to pancreas cancer for use as surrogate endpoints in intervention trials for early detection and cancer prevention. The EDRN is a collaboration of federal, academic, and private research institutes that bring together basic scientists, clinicians, technology developers, epidemiologists, and other health professionals to initiate clinical studies in a streamlined process to identify and validate biomarkers for early detection and appropriate cancer prevention approaches.

The goals of the workshop were to:

- Identify gaps in knowledge about how nutritional status and the use of bioactive food components may affect pancreatic carcinogenesis.
- Develop a research agenda to illuminate key interactions between dietary factors and genetic variables, including identification of candidate biomarkers that are indicative of disease and nutritional risk.

Much remains to be learned about pancreatic cancer etiology and pathogenesis or potential interventions for disease prevention. Although there have been epidemiological and mechanistic studies examining risk factors for pancreatic cancer, there is little consistent or strong evidence to provide a cohesive prevention strategy for this cancer. Epidemiological studies suggest that age, smoking, meat, fat, and carbohydrate consumption, particularly a diet high in glycemic load, may be associated with pancreatic cancer. Furthermore, study findings support the idea that glucose intolerance, physical inactivity, and positive energy balance may play a major role in pancreatic carcinogenesis. "Heavy" alcohol intake also has been reported to increase the risk of pancreatic cancer, while fruit and vegetable intake may decrease risk.

This Executive Summary provides highlights of speaker presentations and salient points covered during the discussion following each session of the workshop and concludes with a compilation of recommendations for future research.

II. BACKGROUND ON PANCREATIC CANCER

A. General Background Information on Pancreatic Cancer

The pancreas is one of the major organs of metabolic function in the human body, and adenocarcinoma of the pancreas is one of most catastrophic and least understood of cancers. Pancreatic cancer accounts for only 2 percent of all newly diagnosed cancers in the United States each year, but for 5 percent of cancer mortality; only 20 percent of patients survive for more than 1 year.

Evidence from clinical studies indicates that the development of pancreas cancer progresses over many years before symptoms appear. The majority of pancreatic cancers originate in the ductal epithelial cells of the exocrine portion of the pancreas. There is, however, extensive interaction between the endocrine and exocrine portions of the pancreas during pancreatic carcinogenesis. Malignancies rarely arise from the acinar cells that form the bulk of the pancreas and contain zymogen granules that store pancreatic enzymes. Islet cell carcinomas, which account for less than 2 percent of all pancreatic cancers, have a better prognosis than pancreatic ductal cancers. Pancreatic cancer is usually at the metastatic stage when symptoms present, making early diagnosis treatment difficult. Therefore, earlier diagnosis and prevention are key areas of research for reducing the high mortality from this disease.

There are racial/ethnic/gender disparities within the United States population in the incidence/mortality rates of pancreatic cancer. Men have higher incidence and mortality rates for pancreatic cancer than women in each racial/ethnic group. Incidence and mortality rates are approximately 50 percent higher for African Americans than for white Americans. Hispanic and Asian Americans, however, have lower rates than whites. Incidence and mortality rates for other racial and ethnic groups in the United States are difficult to compare with African Americans and white Americans because data are generally insufficient. Age, among all racial and ethnic groups in the United States, is positively associated with increased risk of pancreatic cancer, with the median age of diagnosis being approximately 70 years. Graphs showing incidence and mortality for specific racial and ethnic groups are available at the NCI's Surveillance, Epidemiology, and End Results (SEER) Web Site at: http://seer.cancer.gov/.

B. Molecular Aspects

The molecular milestones in the development of neoplastic pancreatic diseases are incompletely understood. Hulst, in 1905, described a number of physiologic patterns in the pancreatic epithelium that appeared to represent a progressive model of pancreatic cancer (See Figure: Pancreatic Intraepithelial Neoplasia (PanIN) System). Modern-day pathologists have adapted and adopted this basic model as a tentative classification schema designated the Pancreatic Intraepithelial Neoplasia (PanIN) system to describe stages of development of ductal carcinomas in the pancreas. The PanIN system has three grades that are characterized by tall mucinous

epithelium (PanIN-1A), papillary infolding of this epithelium with fibrovascular stromal cores (PanIN-1B), moderate dysplasia with nuclear enlargement and pseudostratification (PanIN-2), and severe dysplasia of ductal epithelium (PanIN-3). (Consult the PanIN website http://pathology.jhu.edu/pancreas_panin/ for more complete descriptions.) Clonal molecular changes, e.g. *K-ras* mutation, characteristic of pancreatic ductal carcinoma occur in many PanIN-1 lesions. PanIN physiologic changes may occur for as long as 15 to 20 years before the diagnosis of carcinoma, which explains the critical role of early detection and prevention as the most promising strategy for reducing mortality from pancreatic cancer. Once symptoms arise, metastasis generally has occurred with the resulting unusually high rate of mortality from pancreatic cancer.

Alterations in genes and gene expression patterns have been described for pancreatic cancer in each of the PanIN stages (See Figure: Pancreatic Intraepithelial Neoplasia (PanIN) System). Activation of the *k-ras* oncogene, silencing of tumor suppressor genes such as *p16, MADH4, p53* and other genes, shortening of telomeres, alterations in DNA methylation, amplification of certain oncogenes, overexpression of growth factors, and inactivation of DNA mismatch repair genes play key roles in pancreatic cancer pathogenesis. Multiple genetic changes identified in invasive pancreatic cancer also are present in pre-malignant PanINs, indicating an opportunity for research on prevention and early detection. However, there is a lack of consensus about the direct relationship between bioactive food components or nutritional status and genetic or epigenetic events that are associated with the development of pancreatic cancer.

Most early lesions contain *k-ras* mutations, which are seen in all PanIN stages and often are considered a marker of pancreatic neoplasia. The *k-ras* activation by point mutations at codon 12 is present in >90 percent of ductal pancreatic tumors. Oncogenic *ras* induces gastrin gene expression via activation of the *Raf-MEK-ERK* signal transduction pathway. One surprising finding is that *ras* mutations in the tumor do not correlate with cigarette smoking, which is the most consistently observed environmental risk factor for pancreas cancer. Loss of tumor suppressor genes, which occurs at later stages in the PanIN classification system, can facilitate rapid growth of a transformed clone. Tumor suppressor genes commonly silenced include: *p16*, *p 21*, *p53*, *MADH4*, and *BRCA2*. Overexpression of growth factors, including EGF, IGF1, and TGF-α are also frequent findings in pancreatic cancer studies. Moreover, inactivation of genome

maintenance genes may lead to the loss of fidelity of DNA replication, because errors are not corrected.

C. Dietary and Other Risk Factors for Pancreatic Cancer

Family associated pancreatic cancer, which may or may not be inherited, accounts for 10-15 percent of pancreatic cancers and can occur because of chance clustering, environmental causes, or by high penetrance genes, low-penetrance genes, or polymorphisms. Based on studies in Ashkenazi Jews, the *BRCA2* gene may account for 7-10 percent of pancreatic cancers. Other, less common genetic causes of pancreatic cancer include mutations in the serine protease *PRSS1* gene- known as cationic trypsinogen and accounts for less than 1 percent of pancreatic cancer in families with a form of acute pancreatitis; an autosomal dominant polyposis disorder known as Peutz-Jeghers syndrome (PJS), which is associated with less than 1 percent of cases; and mismatch repair genes, which account for less than 3 percent of cancers of the pancreas in the United States. Individuals with inherited susceptibilities to pancreatic cancer are likely to have high risk ratios that may obfuscate any independent or synergistic risk from nutritional influences in observational studies. Ascertainment of genetically susceptible persons may be essential to identify statistically valid associations between diet and pancreatic cancer in an observational setting.

Most studies of etiological factors in pancreatic cancer have been epidemiological case-control studies. Few prospective diet studies exist, with most reporting on less than 100 cases. Results from these epidemiological studies have identified various probable and possible risk factors for pancreatic cancer.

Probable risk factors include:

- Cigarette smoking
- Longstanding diabetes
- Chronic and hereditary pancreatitis.
- Family History of pancreatic cancer

Possible risk factors for pancreatic cancer include:

- Noncigarette tobacco use (cigars, pipes, and smokeless tobacco- nitrosamines and aromatic amines)
- Other medical conditions (gallbladder disease/cholecystectomy, gastrectomy/peptic ulcer).
- Occupational exposures (e.g., organochlorine compounds [DDT, PCBs] and chlorinated hydrocarbon solvents)
- Heavy alcohol consumption
- Low socioeconomic status
- Dietary factors (e.g., infrequent intake of vegetables, positive energy balance, and high BMI)
- High caloric intake and frequent meals per day

There have been relatively few observational studies exploring possible dietary risk factors for pancreatic cancer. According to the 1997 American Institute for Cancer Research/World Cancer Research Fund (AICR/WCRF) review, dietary factors may account for 35 percent of pancreatic cancer, although for most dietary constituents, overall findings have been equivocal. Recent case-control studies conducted by NCI investigators have shown that men and women in the highest quartile of BMI have a 50 percent increased risk of pancreatic cancer; the highest quartile for caloric intake increased risk 40 percent in men and doubled the risk in women. Moreover, a significant interaction between BMI and caloric intake was found; subjects above the median for both BMI and caloric intake had a 70% increase.

An increased risk for pancreas cancer among people with longstanding diabetes has been observed; this association was found to be independent of insulin use. Data on risk by BMI quartile and diabetes status indicated that for people with no history of diabetes, there was a positive gradient in risk with increasing BMI. Among those who had diabetes, however, BMI was not related to pancreatic cancer risk. This observation is consistent with the hyperinsulinemia hypothesis. In people who do not have diabetes, high BMI is associated with insulin resistance. This is not true in people with diabetes, where there is no association between insulin levels and BMI; rather, insulin levels reflect impaired beta-cell function and hyperglycemia.

Physical activity affects risk of obesity and diabetes but relatively little is known about how it might be related to pancreatic cancer risk. The results of the few studies that assessed physical activity and pancreas cancer indicate that there may be an inverse association between moderate physical activity and the risk of pancreas cancer, particularly in those in the highest tertile of BMI.

Few epidemiological studies have addressed the relationship between carbohydrates and pancreatic cancer. A multi-center, case-control study of subjects from five European countries demonstrated that the highest intake of carbohydrates resulted in a 2.57-fold risk in pancreatic cancer. Analysis of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study indicated a significant inverse association between pancreatic cancer and overall carbohydrate intake. The results of a small number of studies of intake of specific carbohydrates have found an inconsistent association between sucrose intakes and pancreatic cancer; one case-control study among women detected an increased risk related to the amount of sucrose added to foods.

Dietary data from the prospective cohort Nurses' Health Study (NHS) have been examined for the relationship to pancreas cancer risk. Results indicated that glycemic load, carbohydrate, sucrose, and fructose intakes were not significantly correlated with pancreas cancer risk (See section on Oxidative Stress). When the data were stratified by BMI and glycemic load, however, results indicated that there was an increase in pancreas cancer risk with higher carbohydrate, sucrose, and fructose intakes; the association between physical activity, glycemic load and pancreatic cancer risk was not, however, significant. Data analysis of a large prospective cohort of men, however, did not suggest an association between glycemic index and pancreas cancer (Note: unpublished findings mentioned at the meeting).

Interestingly, when the effects of both BMI and physical activity were assessed for associations with pancreas cancer in the NHS cohort, results indicated that individuals in the highest BMI/lowest physical activity group had the highest risk of pancreas cancer. This was especially true among women with high glycemic load or fructose intake. These findings support the hypothesis that impaired glucose metabolism plays a role in the pathogenesis of pancreatic cancer and that a diet high in glycemic load may increase the risk of pancreatic cancer among individuals who already have an underlying degree of insulin resistance. This is a young cohort

and it is possible that these data will strengthen over time. Clearly, other investigators need to examine the association between glycemic index and pancreas cancer using different cohorts before conclusions can be made on this issue.

D. The Metabolic Hypothesis for Pancreatic Cancer

Advances in genomics and proteomics have created parallel strategies for examining major categories of biological molecules. An understanding of the influence of the metabolome, the key substrates, products and intermediates of biochemical pathways on which proteins act, is now emerging. This is a critical area of research because differing levels of metabolites in body cells, tissues, and fluids can be closely linked to normal and aberrant phenotypes.

Pancreatic tumor cells are characterized by poor differentiation and a specific high-glucose utilizing phenotype. Glucose metabolism supplies the primary carbon source for nucleic acid, lipid and amino acid biosyntheses. Biologically based mass spectrometry techniques have been used to document that proliferating tumor cells preferentially use glucose to synthesize the ribose 5-phosphate required for nucleic acid synthesis. This illustrates that the metabolic enzymes that regulate flow of glucose through the oxidative reactions of the pentose pathway may mediate pancreatic carcinogenesis. Thus, investigation of the metabolomic profiles of tumor cells should be integral in the study of the effects of energy and dietary macronutrients on pancreatic cancer.

Metabolic profiling is being investigated to define pancreatic phenotypes associated with normal and neoplastic cells. This strategy has been used to identify certain changes in metabolic phenotypes induced by TGF-β, and tumor growth-inhibiting phytochemicals such as genistein or novel synthetic antileukemic drugs such as STI571 (Gleevec). For example, experiments using glucose isotope tracers have investigated protein synthesis through the steps of pentose production, the Krebs cycle and other pathways. In addition, evidence indicates that intermediary metabolic enzymes, such as those involved in substrate flux through metabolic pathways and the contribution of substrates to macromolecule synthesis, are associated with regulation of growth-signaling pathways that may promote malignant cell transformation. It may be possible to develop these intermediary metabolic enzymes as nongenetic novel targets for cancer chemoprevention or therapeutic interventions.

E. Preclinical Studies on Pancreatic Cancer

Experimental data suggest that some intraductal papillary tumors require many years to develop. Diet is likely to influence this process because of the metabolic functions of the pancreas.

Data from experimental and observational studies suggest that high levels of dietary fat may increase risk and higher consumption of fruits and vegetables may decrease the risk of pancreatic tumors. One of the difficulties of assessing diet and cancer is that humans consume different foods at different times of their lives. Therefore, experimental models should be an important aspect of pancreatic cancer research because it is easier to expose cells and animals to modifying factors that may influence cancer risk. Further development of other experimental animal models, either chemically- or non-chemically-induced and xenografts, are essential for clinical studies.

A model for pancreatic-duct carcinoma in Syrian golden hamsters has been utilized to investigate the effect of exposure to the carcinogen N-nitrosobis(2-oxopropyl) amine (BOP). In one experiment, a single dose of BOP resulted in a low percentage (~9-18%) of animals with pancreatic intraductal or invasive adenocarcinomas. In a second experiment, 59-66% of hamsters developed pancreatic carcinomas after three weekly treatments of the same dose of BOP. No hamsters developed ductal hyperplasia if early exposure to an initial BOP dose was omitted. Possible mechanisms for promotion in this model include increased cell turnover during recovery from pancreatitis that accelerates carcinogenesis and hypomethylation of DNA that alters gene expression in a manner that promotes carcinogenesis.

Experimental models for pancreatic cancer have been developed in rats and hamsters to investigate the effect of the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). NNK administration causes a low incidence of pancreatic cancer when given to adult rats, but results indicate that a high incidence of pancreatic cancer develop in the offspring of hamsters that were transplacentally exposed to a single dose of NNK and ethanol throughout the gestation period. The β-blocker propranolol reduced the incidence of tumors, suggesting that andrenergic receptors were involved in cancer development. Radio-receptor assays in Chinese

hamster ovary (CHO) cells transfected with the human β_2-adrenergic receptor gene or the human β_1-adrenergic receptor gene have been studied to investigate the role of high affinity binding of NNK to both receptors. These data unequivocally document the binding of NNK as a competitive ligand to β_1-and β_2-adrenergic receptors. Additional studies suggest that there may be a synergism of the β_2-adrenergic pathway with the epidermal growth factor (EGF) pathway, although details of signaling events have not been elucidated.

The hamster pancreatic cancer model has been used to show that most pancreatic adenocarcinomas develop from stem cells within islets, which have the potential to differentiate or become malignant, possibly due to their high levels of drug-metabolizing enzymes and high proliferation rate. The relevance of the hamster model, however, is a matter of debate because islet (*versus* intraepithelial) ductal metaplasia has not been identified in human tissue. Dietary studies in hamsters suggest that the promoting effect of a high-fat, high-protein diet on carcinogenesis within the islet cells is unrelated to the energy intake, but is related to the effect of the diet on cellular replication. Saturated, monounsaturated, and omega-6 polyunsaturated fatty acids may increase the incidence of several cancers, including pancreatic cancer; however omega-3 fatty acids appear to have an inhibitory effect on cancer. In the hamster model, high-fat diets cause insulin resistance and islet cell hyperplasia. Islet cell proliferation is likely a major factor in promoting the effect of high-fat diets on pancreatic carcinogenesis.

Folate is abundant in the pancreas of rats. Studies of folate deficiency and pancreatic function have reported that the ratio of S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH) is significantly reduced in rats fed a folate-deficient diet. The folate-deficient animals also had more immature secretory granules in the pancreas than controls and pancreatic amylase secretion was reduced. (See Methylation section for additional discussion)

The use of porcine pancreatic enzymes (e.g., trypsin and amylase) as a treatment for experimentally induced pancreatic cancer in a mouse model suggests that survival can be extended in treated mice compared to controls. In addition, pathologic studies showed that tumor weight and volume were lower in the treated mice compared to the controls. These preliminary findings suggest that survival was due to improved nutrition; however, other factors may have contributed to these results. These data are especially noteworthy because a recent

pilot study of proteolytic enzyme treatment in 11 patients with confirmed pancreatic adenocarcinoma suggested that aggressive nutritional therapy, combined with large doses of pancreatic enzymes, significantly increased survival of patients with inoperable pancreatic adenocarcinoma.

III. METHYLATION

DNA methylation, gene expression and pancreatic cancer

The relationship of DNA methylation to cancer is complex. Global hypomethylation, accompanied by region-specific hypermethylation, is a common characteristic among tumor cells. Methylation-mediated abnormalities of gene expression in cancer include overexpression and loss of expression. Overexpression of proto-oncogenes, chromosomal translocations, and loss of imprinting may be influenced by DNA hypomethylation; loss of expression may occur by promoter hypermethylation with subsequent transcriptional silencing of tumor suppressor and mismatch repair genes in many cancers.

Hypermethylation of CpG islands in the 5'flanking region of genes has been identified in various cancers. Such promoter hypermethylation has been correlated with the loss of expression of numerous genes in pancreatic cancer including *p16, ppENK, SOCS-1, TSLC-1, SPARC* and others. Methylation of many of these genes has been observed in precancerous pancreatic neoplasms. Experimental studies of gene expression patterns in pancreatic cancer cell lines have identified many genes overexpressed in pancreatic cancer including *14-3-3 δ*, *Claudin-4*, *PSCA*, *S100A4, S100P, MMP-7, IL-8, CD44*, and *mesothelin*. Many of these genes are normally methylated and not expressed in normal pancreatic epithelium and undergo gene specific hypomethylation in conjunction with overexpression in pancreatic cancer. More studies are necessary to gain an understanding of their connection to methyl availability and possible site-specific DNA hypomethylation events.

Preclinical studies: one-carbon metabolism and pancreatic cancer

Biological plausibility for an association between methyl-deficient diets and pancreatic cancer include: a high-specific requirement for methyl donors—the pancreas contains a high concentration of folic acid derivatives, including 5-methyltetrahydrofolate (MTHF), which is the

product of the reaction catalyzed by methylenetetrahydrofolate reductase (MTHFR); inhibition of methylation reactions resulting in pancreatitis; the understanding that methylation is needed for exocrine pancreatic secretion; and reduced pancreatic enzyme secretion resulting in an increase of the peptide hormone cholecystokinin (CCK). The available cell culture and animal experimental evidence as well as epidemiological evidence support the need for more probing studies on the relationship(s) between diet, DNA methylation, and pancreatic cancer.

Studies using an acinar cell differentiation model indicate that methionine is required for acinar cell survival, growth, and differentiation. Choline alone does not fully support acinar cell differentiation; however, choline with homocysteine supports acinar cell differentiation. Studies in embryonic rat pancreas show that a basal level of methionine (30 mg/L) is required for cells to grow *in vitro*; for differentiation to proceed normally, *in vitro*, additional methionine (50 mg/L) is required. SAM and choline are both methyl donors that can substitute to some degree for the incremental methionine requirement to support differentiation. These findings suggest that methyl groups are necessary for differentiation in this model.

Transdifferentiaton (metaplasia) of pancreatic cells into hepatocytes has been investigated in hamster and rat cell lines. A methyl-deficient diet appears to stimulate transdifferentiation in acinar cells, ductal cells, endocrine cells, intermediate cells, or stem cells in lobular tissue, often near islets. Copper depletion, ethionine treatment/methionine deficient diet, peroxisome proliferators, and pancreatic carcinogens (BOP, 4-HAQO, MNCO, MNNG) also have been shown to increase transdifferentiation. The appearance of "pancreatic hepatocytes" in rats fed choline-deficient diets, combined with observations of similar cells in hamsters treated with ethionine, provide additional evidence that the lack of available physiological methyl donors contributes to altered differentiation of pancreatic acinar (or stem) cells. Ethionine-induced pancreatitis in rats was characterized by prompt onset of zymogen retention and delayed onset of necrosis and vacuolization. The loss of acinar cells with sparing of islets and ducts was reversed or prevented by the addition of methionine to the diet. Similar observations have been made in the mouse, dog, and guinea pig. The effect of ethionine administration and choline deficiency on pancreatic protein carboxymethylase activity was investigated in mouse pancreas. Feeding an ethionine-containing diet decreased protein carboxymethylase activity, and this effect was

enhanced by simultaneous choline deficiency suggesting that this mechanism may inhibit enzyme discharge from acinar cells.

Glycine N-methyltransferase (GNMT) is abundant in rat liver and pancreas and appears to be regulated by folate availability. An investigation of folate deficiency on pancreatic function in a rat model reported that GNMT activity is increased and the ratio of SAM to SAH is significantly reduced in rats fed a folate-deficient diet. GNMT contains bound 5-methyl tetrahydrofolate, which also acts as an inhibitor of the reaction. When cellular methionine levels are low, SAM levels decrease, but 5-methyl tetrahydrofolate levels increase. The latter inhibits GNMT, leading to the conservation of SAM for critical methylation reactions. Rats fed a folate-deficient diet also had more immature secretory granules in the pancreas than in controls, and pancreatic amylase secretion was reduced.

Investigations using animal models suggest that defective methylation in the pancreas affects pancreatic cell growth, acinar cell differentiation and function, susceptibility of the pancreas to toxicity of ethionine, and the rate of development of pancreatic carcinomas in an animal model of carcinogen induced adenocarcinoma.

Epidemiological Studies: Dietary methyl donors, polymorphic methylation and pancreas cancer
Two prospective epidemiological studies have been conducted to evaluate the association between dietary or nutritional status indicators, other factors known to influence methyl group availability, and pancreatic cancer. In the ATBC study, dietary folate intake was inversely and significantly associated with pancreatic cancer, with the highest quintile having half the risk of the lowest quintile. Moreover, smoking dose and cumulative smoking was positively and significantly associated with pancreatic cancer risk, with the highest quintile having twice the risk of the lowest quintile. By contrast, there were no significant associations between dietary intake of vitamin B12—but investigators did not consider bioavailability of this nutrient for certain subgroups, vitamin B6, methionine, alcohol and risk of pancreatic cancer. In the other ATBC analysis, serum folate was also inversely and significantly associated with pancreatic cancer, with the highest tertile having approximately 50 percent decreased risk compared to the lowest tertile. Furthermore, serum PLP concentrations were inversely and significantly associated with pancreatic cancer risk, with the highest tertile having half the risk of the lowest

tertile. There was no association between serum vitamin B12 (perhaps because serum B12 is neither sensitive nor specific) or total homocysteine concentrations and risk of pancreatic cancer. Smoking was positively associated, but alcohol was not associated.

Polymorphic genes involved in methylation reactions also are the focus of studies to understand the relationship between methyl groups, folate, and cancer. Polymorphisms in the *MTHFR* gene are associated with an elevation in serum homocysteine levels that have been linked to a number of cancer types, but the association varies. A 677C to T variant in this gene encodes a thermolabile enzyme with reduced activity that leads to reduced plasma folate levels and for some cancers this 677T variant is protective, in others it appears to be associated with increased risk. The 677T variant also results in increased levels of 5-10 methylene THF which helps maintain optimal thymidine production. Low thymidine results in uracil misincorporation into DNA. Uracil in DNA is excised by the repair enzyme uracil DNA glycosylase creating transient single-strand breaks in DNA in the process that needs to be repaired. The relationship between *MTHFR* polymorphisms and pancreatic cancer has been studied. A case-control study was conducted to determine the prevalence of *MTHFR* polymorphisms (677-C to T and 1298-A to C polymorphisms in *MTHFR*) in 228 patients with pancreatic adenocarcinoma and in 245 controls. There was no observed effect between MTHFR status and risk of pancreatic cancer. The odds ratio for pancreatic cancer in cases homozygous for the *MTHFR* polymorphism at locus 677 was 1.12; for those with the *MTHFR* polymorphism at locus 1298, the odds ratio was 1.2 for pancreatic cancer. Heterozygosity in either gene did not reach statistical significance (unpublished).

MTHFR polymorphisms occur in an area of the gene where loss of heterozygosity occurs in pancreatic and other cancers. It is possible that the *MTHFR* genotype of an evolving cancer may influence its behavior. Additional studies are needed to determine if the *MTHFR* 677T genotype is more likely to be associated with genetic alterations in pancreatic cancer cells.

IV. OXIDATIVE STRESS

Inflammation and oxidative stress have been implicated in pancreatic cancer etiology. Inflammatory cytokines, reactive oxygen species (ROS), and mediators of inflammatory

pathways, such as cyclooxygenase-2 (COX-2) and Nuclear Factor kappa B (NFkB), are associated with oncogene expression, silencing of tumor suppressor genes, and affect the cell cycle, all of which may facilitate pancreatic carcinogensis. Mediators of the inflammatory response may also induce genetic damage, cell proliferation, and inhibition of apoptosis in the pancreas. Because ROS contribute to the inflammatory process, evaluating the potential cancer protective effects of dietary antioxidants is a logical step in this area of research.

Antioxidants have been used alone or in combination with other agents, including Peptide YY (PYY), a gut hormone that suppresses pancreatic exocrine function and lowers insulin secretion. PYY, a 36-amino acid peptide secreted by endocrine cells of the distal bowel postprandially, belongs to the pancreatic polypeptide family. It inhibits growth of many cancers *in vitro* and *in vivo*. Experimental studies have shown that PYY and vitamin E individually inhibit the growth of pancreatic cancer cells; however, combining PYY and vitamin E results in a greater inhibition than either alone. Possible mechanisms include decreased EGF receptor expression, decreased intracellular cAMP, increased apoptosis, or decreased NFkB activity.

NFkB is a nuclear transcription factor that is activated by many stimuli. When activated, it affects target genes that exert its varied functions, including enhancing secretion of inflammatory cytokines. The role of inflammatory cytokines in pancreatic cancer offers a plausible mechanism for prevention. For example, researchers have utilized CAPAN-1 and CAPAN-2 cells, well-differentiated human pancreatic adenocarcinoma cells that retain multiple histologic and biochemical markers of differentiation, to investigate the influence of cytokine production (i.e., IL6 and IL8) on pancreatic cancer development. IL6 and IL8 enhance pancreatic cancer growth and metastasis. Additional studies in CAPAN-1 and CAPAN-2 cells indicate that antioxidants (i.e., vitamin E and butylated hydroxyanisole [BHA]) can modify the cytokine profiles of pancreatic cancer cell lines. Antioxidants inhibited both NFkB activation and IL-6 and IL-8 secretion in the CAPAN-2 cell line, but failed to suppress them in the CAPAN-1 cell line. These results suggest that cytokine expression in these cells may be more complex than previously anticipated, and could involve mechanisms other than NFkB activation.

In pancreatic cancer, there frequently is an overexpression of phospholipase A_2, which cleaves arachidonic acid (AA) from membrane phospholipids, with a concomitant overexpression of

AA-metabolizing enzymes of the COX and lipoxygenase (LOX) families. For example, COX-2 is overexpressed in the atrophic acinar cells, hyperplastic ductal cells, and islets cells in most patients with chronic pancreatitis, but not in normal pancreatic tissues. Agents that block COX enzymes have been shown to significantly inhibit pancreatic cancer cell growth and to induce apoptosis. Other data suggests that a selective COX-2 inhibitor may slow the growth of human pancreatic cancer through changes in gene expression that favor cell cycle arrest. Fewer studies of LOX enzymes have been conducted, but they indicate that expression of both 5-LOX and 12-LOX occurs in pancreatic cancer cell lines and cause increased proliferation, which is blocked by chemoprevention agents or antisense oligonucleotides.

It is noteworthy that high levels of exposure to reducing sugars, such as glucose and fructose, may increase oxidative stress in pancreatic cells. Fructose increases cellular peroxide levels and lipid peroxidation in hamster islet tumor (HIT) cells. Fructose has also been shown to suppress the expression of glutathione peroxidase (GPx) mRNA and, in addition, causes inactivation of GPx in HIT cells. The impact on GPx suggests a mechanism by which fructose induces oxidative stress. The role of fructose and its interactions with nitric oxide, which also increases intracellular peroxide levels in HIT cells, may represent an important mechanism affecting apoptosis in proliferating pancreatic tumors. Pancreatic inflammation, mediated by cytokines, reactive oxygen species, and upregulated pro-inflammatory pathways, may play a key role in the early development of pancreatic malignancy. New research and techniques focused on the role of inflammation in the development of pancreatic cancer may assist in the development of prevention strategies for this cancer.

V. RECOMMENDATIONS

Workshop participants at the conclusion of the speaker presentations developed the following list of recommendations. They are listed by topic to reflect promising areas of research and are not listed by priority.

A. Epidemiological Studies

There is a need for epidemiological studies in large and diverse human populations to further assess the following areas of interest:

- Roles of dietary components in pancreatic cancer etiology
    - Caloric intake and meal frequency
    - Carbohydrates (e.g., fructose, glucose, and sucrose) and high glycemic index
    - Lipids, fatty acids ratios, and saturated and trans-fatty acids
    - Antioxidants (e.g., vitamins, beta carotene, ascorbic acid, and tocopherols)
    - Flavonoids and polyphenols
    - Selenium
    - Dietary participants in methylation reactions or methyl donors (e.g., folate, choline, vitamin B6, and vitamin B12)
    - Combined effect of folate restriction with ETOH (and other combinations)
    - Phytochemicals
    - Method of food/meat preparation or (i.e., smoked foods, grilled meats)
- Genetic polymorphisms that affect carcinogen metabolism or epigenetic mechanisms (e.g., cytochrome P450, methylenetetrahydrofolate reductase)
- Etiologic roles of and interactions between various risk factors: hyperinsulinemia, diabetes mellitus, hereditary pancreatitis, chronic pancreatitis, high BMI, obesity, energy balance and alcohol.

B. Preclinical Studies

Areas of research that have shown promise in our understanding of nutrition and pancreatic cancer, which deserve further study include:

- Antioxidants, ROS, and oxidative stress
- Methyl donors and changes in methylation patterns, including effects on SAM:SAH ratios in cells
- Insulin resistance and associated factors
- COX-2 and the arachidonic acid cascade
- Peroxisome Proliferator-Activated Receptors (PPARs) contributions to regulation of cellular growth and differentiation, inflammation and the development of diabetes.

- Examine pancreatic juice and pancreatic lesions for *k-ras* and other gene biomarkers, methylation markers, mitochondrial DNA markers.
- Animal models
    - Development of a pancreatic ductal adenocarcinoma that could then be made available through the Mouse Models of Human Cancer Consortium (URL link to MMHCC: http://emice.nci.nih.gov/)
    - Hamster BOP model
    - ETOH/NNK intergenerational model
    - Transgenic model of acinar cell cancer
    - Nude mouse model using xenografts

C. Methodological Procedures

There is a need to develop standardized procedures for collecting and analyzing biological fluids, developing dietary instruments, and assessing immunologic factors in studies of pancreatic cancer. If possible, existing biological repositories should be considered as resources to provide samples for study.

D. Biomarker Profiling and Early Detection

The unfavorable diagnostic stage distribution and limited stage-specific survival contribute to poor overall survival of pancreas cancer. However, the relative improvement in survival associated with earlier stage pancreatic cancer motivates the search for effective early detection and screening. Of particular potential value are molecular markers detectable in surrogate biological specimens obtained non-invasively, most commonly peripheral blood samples. There is a need to develop biomarkers for preclinical and clinical pancreatic cancer.

Profiling: The primary goals of profiling studies are: 1) to determine the utility of this approach to usefully discriminate between sera of pancreatic cancer cases, chronic pancreatitis patients, and controls; and 2) to evaluate the performance characteristics (sensitivity and specificity) of this method in detecting pancreatic disease. In addition, serum expression profiles from pancreatic cancer patients collected prospectively following initial diagnosis and surgical

resection will assist in investigating the temporal stability of the diagnostic profiles and may help in evaluating the utility of this methodology to provide an early marker of pancreatic cancer recurrence. Areas of promise for developing biomarkers include:

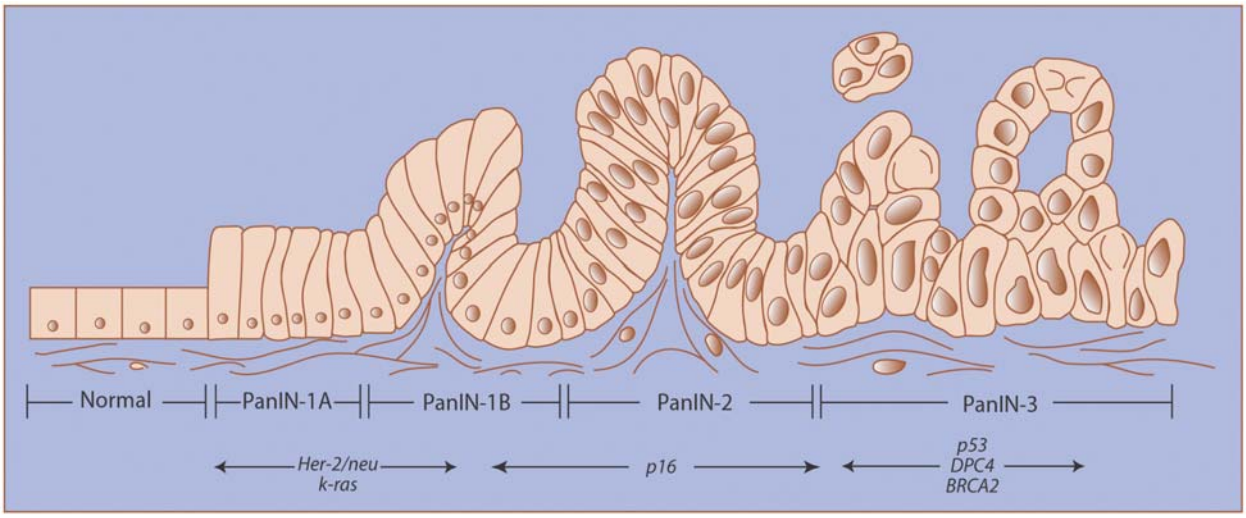
- Proteomic profiling (tissue panel, serum panel, cell lines, and inflammatory cytokines); including use of 2-Dimensional gels and mass spectrometry for profiling
- Metabolic profiling
- Methylation profiling
- Availability of serum and tissue samples from Specialized Programs of Research Excellence (SPOREs) (http://deainfo.nci.nih.gov/flash/pancreaticP50.htm)
- Gene expression profiling

E. Modulators of Pancreatic Cancer

The following areas of research may hold promise for increasing our understanding of pancreatic cancer:

- Immunological parameters/factors (T-cells, B-cells and natural killer cells (NK))
- Inflammatory factors, including cytokines
- Hormones and trophic factors
- Further testing of porcine pancreatic enzyme therapy in patients with known pancreatic cancer

Pancreatic Intraepithelial Neoplasia (PanIN) System



(Hulst SPL. Virchows Arch B 1905;180:288-316)