Distinctive Heavy Metal Composition of Pancreatic Juice in Patients with Pancreatic Carcinoma

Patricia E. Carrigan,[1] Joseph G. Hentz,[1] Gwyneth Gordon,[3] Jennifer L. Morgan,[4] Massimo Raimondo,[2] Ariel D. Anbar,[3,4] and Laurence J. Miller[1]

[1]Department of Molecular Pharmacology and Experimental Therapeutics and Mayo Clinic Cancer Center, Mayo Clinic, Scottsdale, Arizona; [2]Division of Gastroenterology and Hepatology, Mayo Clinic, Jacksonville, Florida; and [3]School of Earth and Space Exploration and [4]Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona

Abstract

Epidemiologic studies have shown the health risks of exposure to cigarette smoke and air pollution, with heavy metal composition implicated as contributing to both. Environmental exposure to cigarette smoke has been epidemiologically associated with pancreatic cancer, but the pathophysiologic basis for this is not yet clear. In the current work, we have used inductively coupled plasma mass spectrometry to quantify the metal composition of pancreatic juice collected in response to secretin stimulation in successive patients evaluated for abdominal pain (35 with pancreatic cancer, 30 with chronic pancreatitis, and 35 with normal pancreas). Indeed, metal composition of pancreatic juice was distinctive in patients with pancreatic cancer relative to those without such a cancer. The metal concentrations that were found to have the strongest association with pancreatic cancer were chromium, selenium, and molybdenum, with 1 SD increases in the concentrations of each associated with substantial increases in the odds of having pancreatic cancer relative to those in patients with normal pancreas (210%, 160%, and 76%, respectively). Of note, elevations in concentrations of chromium and selenium did not correlate in individuals, whereas those having a 1 SD increase in the sum of the concentrations of these two metals in their pancreatic juice had a 480% increase in the odds of having pancreatic cancer. Elevations of nickel and zinc correlated with elevated chromium in individuals, with each of these metals known to be present in cigarette smoke, whereas other recognized metal components of cigarette smoke were not elevated. An understanding of why these metals are elevated in pancreatic juice and what effects they might have on pancreatic cells may have important implications for the diagnosis, treatment, and even prevention of pancreatic cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(12):2656–63)

Introduction

Pancreatic cancer is one of the most aggressive types of cancer, having an extremely poor prognosis. Despite being the 14th most common type of cancer, it represents the fourth most frequent cause of cancer death. A genetically defined basis for pancreatic cancer with a familial incidence represents <10% of cases (1-3), with the majority of cases being incidental, without a clear etiology. Dietary and environmental factors have been implicated in playing a role in some of these. Chronic pancreatitis, including alcohol-related disease, and cigarette smoking are recognized associations, with each increasing the incidence of this cancer by ~2-fold (4, 5). Indeed, ~30% of patients with pancreatic cancer have a history of smoking (6). However, unlike lung cancer where smoking is associated with an anatomically understandable direct delivery of carcinogens, the mechanism of association of smoking with pancreatic cancer is less well defined.

Cigarette smoke contains >4,000 chemical components, including over 30 heavy metals (7-9). Although smoking is a cause of pancreatic cancer, it remains uncertain which compounds in cigarettes might be responsible for this increased risk and how they might be delivered to the pancreas (4, 5, 10). In the current work, we have examined whether the heavy metals known to be present in cigarette smoke that have been associated with lung carcinogenicity (11-13) can also reach the pancreas.

It is well recognized that carcinogenesis in solid organs often represents a multistep process in which sequential genetic alterations favor the selection and proliferation of a clone of cells that develop into a tumor. Multiple reports support the concept that metals like nickel, chromium, cobalt, and arsenic can interfere with key steps in repairing DNA damage (14-18). Some metals may also stimulate cell proliferation, by activation of early response genes or by interference with genes down-regulating cell growth and senescence (19). These two effects can be synergistic in the development of a neoplasm. Other potentially important effects of metals include actions on signaling cascades (20) and even direct genotoxic effects, such as those stimulating G-T

Received 4/11/07; revised 8/2/07; accepted 9/24/07.

Grant support: The Canary Foundation/American Cancer Society postdoctoral fellowship (P.E. Carrigan), Mayo Clinic/Arizona State University Pilot Grant (P.E. Carrigan, A.D. Anbar, and L.J. Miller), and the Fiterman Foundation (L.J. Miller).

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Requests for reprints: Laurence J. Miller, Department of Molecular Pharmacology and Experimental Therapeutics and Mayo Clinic Cancer Center, Mayo Clinic, 13400 East Shea Boulevard, Scottsdale, AZ 85259. Phone: 480-301-6650; Fax: 480-301-6969. E-mail: miller@mayo.edu

doi:10.1158/1055-9965.EPI-07-0332

transversion in K-RAS codon 12 that is present in the majority of pancreatic cancers (21).

In the current study, we have quantified heavy metals in pancreatic juice in a sequential series of patients investigated for abdominal pain felt to be consistent with pancreatic origin, with a subset of these patients found to have pancreatic cancer. This includes a spectrum of pancreatic pathology, ranging from normal to chronic pancreatitis to pancreatic cancer, providing not only informative cases of pancreatic cancer, but also highly useful reference groups. Indeed, the unique metal profiles found to be present in the pancreatic juice of cancer patients might provide important new insights into mechanisms of pancreatic carcinogenicity.

Experimental Procedures

Human Subjects. Patients entered the study after providing written informed consent, as required by the Mayo Clinic Institutional Review Board. Pancreatic juice was collected in 2003 and 2004 from 118 successive qualified and consenting patients who were being evaluated for abdominal pain compatible with being of pancreatic origin. Only patients over 18 years of age were included, with those having had a previous history of pancreatic or gastric surgery and those unable to tolerate upper gastrointestinal endoscopy excluded from the study. Less than 10% of the 130 patients who were evaluated for pancreatic pain during this period were disqualified based on entry criteria or refused entry (nine patients declined participation or did not have endoscopic procedures, two patients had Billroth II gastric resections, and one patient could not safely be sedated for the procedure), with none of these later found to have pancreatic cancer. This cohort was used in previous studies of pancreatic juice cytokine composition and for focused evaluation of zinc (22, 23). Samples from 18 of the original cohort randomly spanning all three diagnostic groups had been depleted and were no longer available for the current study. Of the 100 patients entering this study, 35 had pancreatic cancer and 65 had no pancreatic cancer, with 30 of these having nonmalignant pancreatic pathology (chronic pancreatitis), and 35 having no evidence of intrinsic pancreatic disease (normal endoscopic retrograde cholangiopancreatography). All of the pancreatic cancers were pathologically documented by positive cytology and/or histology. Fifty-one percent of the patients in both groups were current or former smokers. Unfortunately, the number of patients who had pancreatic cancer who were active smokers was quite small (five patients), providing little power for the direct comparison of smokers versus nonsmokers with pancreatic cancer.

Pancreatic Juice Collection. Pancreatic juice was collected as described previously (22, 23). During upper gastrointestinal endoscopy, a 7-French plastic catheter (Wilson Cook) was placed in the second portion of the duodenum. This was used to collect pancreatic secretions stimulated by i.v. infusion of porcine secretin (SecreFlo; Repligen). Samples were immediately frozen on liquid nitrogen and were stored at −80°C until mass spectrometric assays could be done.

Sample Preparation and Mass Spectrometry. Before use, all centrifuge tubes and pipette tips were cleaned by sequential soaking in a mild detergent for 1 week, 3.2 mol/L nitric acid for 1 week, and 2.4 mol/L hydrochloric acid for 1 week. Pancreatic juice samples were thawed on ice, divided into aliquots, placed into acid-cleaned Teflon reaction vessels (Savillex Corporation), and dried on a hotplate in a metal-free, class-10 laminar airflow exhaust hood. Once samples were dry, concentrated, subboiling distilled nitric acid was added to each sample and refluxed at 200°C for 12 h. The nitric acid solution was evaporated to dryness, diluted with 0.32 mol/L nitric acid, and analyzed for metal concentrations using a quadrupole inductively coupled plasma mass spectrometer equipped with a collision cell (Thermo Scientific X Series; Thermo Fisher Scientific, Inc.). A custom, matrix-matched, multielement calibration standard was designed around the measured concentrations of elements from initial sample analyses, and this custom standard was used for all subsequent calibration of elemental concentrations. Check blanks were run every five samples, and procedural blanks were subtracted from the measured concentrations. The internal standard correction factor for each measured element was interpolated from a plasma response curve calculated from a multielement internal standard (Ge, Y, In, and Bi).

To validate our methodology, aliquots from the custom calibration standards were processed in parallel with experimental samples, and run as unknowns on the inductively coupled plasma mass spectrometer to quantify the elemental recovery through the sample preparation protocol. The mean of three replicate standards processed in this manner was within 5% of the known value for 11 elements (Cr, Fe, Mn, Co, Ni, Cu, Zn, Sr, Cd, Ba, and Pb) and within 10% of the known value for Ti and V. The mean for the Al concentration was 29% higher, whereas Se and Mo were lower than the known values (22% and 17%, respectively). The internal analytic uncertainty on three replicate runs was typically better than 2%.

Statistical Methods

Patient Demographics. Demographic information was collected at interview at the time of patient accrual to the study. The mean age and body mass index were compared among groups by using one-way ANOVA. The distributions of sex and smoking history were compared by using the Pearson χ^2 test.

Metal Composition of Pancreatic Juice. The relationship between pancreatic cancer and metal concentrations was quantified by using a general linear model with terms for disease group, age, sex, and smoking history. Smoking history was included as a categorical variable and age was included as a continuous variable. The relationship between pancreatic cancer and metal concentration among patients of like age, sex, and smoking history was also quantified by using multiple logistic regression. Adjusted odds ratios were determined for a difference of 1 SD of the metal concentration. The SDs were estimated by using the sample of patients with normal pancreas. The relationship between pancreatic cancer and multiple metals was assessed by using forward selection.

The correlations between metal concentrations were quantified by using Pearson correlation coefficients. This was done in attempt to identify shared versus

independent origins of the metals in pancreatic juice of individuals. The correlations between metals were determined for all the patients, for those with pancreatic cancer, and for those with normal pancreas. The correlations between metals for the latter two groups were also compared with each other. The correlations between metals among current smokers were similarly compared with those who never smoked. The statistical significance of the comparisons of correlations was calculated by using the Fisher's Z transformation method.

All computations were done using SAS software, version 9.1. P values <0.05 were considered to be statistically significant.

Results

Patient Demographics. The demographics of the patient groups are described in Table 1. As might be expected for patients selected based on diagnostic concern about the possible pancreatic basis of abdominal pain, the demographics of the groups were skewed to reflect the incidence of pancreatic cancer in the general population. Consistent with this, patients in this series who had pancreatic cancer were typical of this disease overall, being older than their counterparts without cancer and exhibiting a male predominance. This type of patient series did provide important reference groups representing the full spectrum of pancreatic pathology, including those with no evidence of pancreatic disease. In this series, the percentage of patients with a history of smoking did not differ between the group with cancer and that without cancer [Δ = 0.01; 95% confidence interval (95% CI), −0.19-0.21]. The percentage of current smokers in the group with pancreatic cancer was not higher than the percentage in the group without pancreatic cancer (Δ = −0.15; 95% CI, −0.30-0.03). Body mass index was not significantly different in the groups with and without pancreatic cancer (Δ = −0.5; 95% CI, −3.4-2.5 kg/m^2).

Metal Composition of Pancreatic Juice. A broad menu of metals was quantified in the pancreatic juice using carefully validated mass spectrometric techniques. The absolute concentrations of the 16 metals and the SDs of these measurements are listed in Table 2. As expected, based on the differential abundance of the metals and on possible differences in individual exposures, concentrations in the pancreatic juice varied considerably from metal to metal and many metals had a broad dispersion of values.

Table 3 shows the adjusted mean concentrations of the metals and the P values for testing whether these concentrations were different in the pancreatic cancer group from those in the group with pancreatitis, those in the group with normal pancreas, and in those in these two noncancer groups together. The metals in pancreatic juice that were found to have the strongest associations with pancreatic cancer were chromium, selenium, and molybdenum. A 14 μg/L (representing 1 SD of the concentration in the normal pancreas group) increase in chromium concentration corresponded to a 210% increase in the odds of having pancreatic cancer relative to that of the group with normal pancreas (odds ratio for this increase was 3.1; 95% CI, 1.23-7.8; odds ratios are listed in Table 4). A 13 μg/L (representing 1 SD) increase in selenium concentration corresponded to a 160% increase in the odds of having pancreatic cancer relative to that of the group with normal pancreas (odds ratio for this increase was 2.6; 95% CI, 0.94-7.1). A 2.2 μg/L (representing 1 SD) increase in molybdenum concentration corresponded to a 76% increase in the odds of having pancreatic cancer relative to that of the group with normal pancreas (odds ratio for this increase was 1.76; 95% CI, 0.82-3.8). Multivariable analysis indicated that the combination of chromium and selenium had the strongest independent relationship with pancreatic cancer as well. A 20 μg/L (representing 1 SD) increase in the sum of chromium and selenium concentrations corresponded to a 480% increase in the odds of having pancreatic cancer relative to that of the group with normal pancreas (odds ratio for this increase was 5.8; 95% CI, 1.55-22).

Associations between the levels of specific metals in individual patients were examined to explore whether they were independent variables or whether they could be related. Interestingly, chromium and selenium concentrations in pancreatic juice of individuals did not correlate with one another (Table 5; r = −0.02; 95% CI, −0.22-0.18). This was not different for current smokers or those who never smoked (Table 6). Based on absence of correlation with each other, these two metals likely come from independent sources, yet both were associated with the prevalence of pancreatic cancer. Of particular interest, the only metal found to correlate with selenium in pancreatic juice was molybdenum, with this association stronger in patients with pancreatic cancer (r = 0.81) than in those with normal pancreas (r = 0.28).

Chromium elevation in pancreatic juice of individuals correlated significantly with elevations of zinc (r = 0.63; 95% CI, 0.49-0.74) and nickel (r = 0.80; 95% CI, 0.72-0.86)

Table 1. Age, sex, body mass index, and smoking history for 35 patients with pancreatic cancer, 30 patients with pancreatitis, and 35 patients with normal pancreas

	Pancreatic cancer	Pancreatitis	Normal pancreas	P
Age (y), mean ± SD	70 ± 10	58 ± 15	48 ± 13	<0.001
Male	23 (66%)	15 (50%)	9 (26%)	0.003
BMI (kg/m^2), mean ± SD	26.9 ± 6.8	26.3 ± 5.3	28.4 ± 8.5	0.45
Smoking				0.18
Never	17 (49%)	12 (40%)	20 (57%)	
Former	13 (37%)	7 (23%)	7 (20%)	
Current	5 (14%)	11 (37%)	8 (23%)	

Abbreviation: BMI, body mass index.

Table 2. Crude mean ± SD metal concentrations in pancreatic juice in μg/L for 35 patients with pancreatic cancer, 30 patients with pancreatitis, and 35 patients with normal pancreas

	Pancreatic cancer	Pancreatitis	Normal pancreas
Ni	21 ± 35	11 ± 17	13 ± 29
Cr	28 ± 73	10 ± 16	9 ± 14
Co	0.81 ± 0.75	0.47 ± 0.47	0.44 ± 0.52
Sr	80 ± 150	34 ± 27	49 ± 66
Cd	1.2 ± 1.3	1.3 ± 1.4	1.1 ± 1.1
Pb	3.6 ± 3.4	1.3 ± 1.5	4 ± 11
Al	86 ± 93	70 ± 110	120 ± 230
Ti	180 ± 180	170 ± 140	150 ± 130
V	0.94 ± 0.84	0.83 ± 0.66	0.70 ± 0.61
Mn	22 ± 20	61 ± 89	46 ± 48
Fe	2,600 ± 6,400	900 ± 1,600	470 ± 480
Cu	270 ± 270	160 ± 120	290 ± 420
Zn	2,400 ± 3,900	1,140 ± 910	1,400 ± 1,400
Se	60 ± 37	51 ± 19	43 ± 13
Mo	5.2 ± 7.9	3.0 ± 1.2	3.1 ± 2.2
Ba	270 ± 670	160 ± 180	190 ± 200
Ni, Cr, Co, Sr, Cd, Pb	140 ± 200	58 ± 44	76 ± 79
Cr, Se	88 ± 77	61 ± 29	52 ± 20

in the same individuals (Table 5A). These associations were significantly stronger in the pancreatic cancer patients than in those with normal pancreas (Table 5B and C). For patients who were current smokers, elevations of nickel and zinc correlated with each other (r = 0.94), whereas this association was missing in the group who never smoked (r = 0.01; Table 6A and B). There was no association of cobalt, lead, or strontium, other metals found in cigarette smoke (9), with the elevated chromium observed in the pancreatic juice of the pancreatic cancer patients. The only other heavy metal significantly associated with elevated chromium was vanadium (r = 0.41; 95% CI, 0.23-0.56). This association was also significantly stronger in the current smokers relative to those who never smoked (Table 6).

Discussion

People may be chronically exposed to potentially harmful metals that are found in environmental pollution (such as cigarette smoke or automobile exhaust), water, soil, and food. Prolonged exposure to metals and their accumulation in tissues may disrupt normal metal homeostasis in the body, thus contributing to the pathogenesis of a number of diseases. However, there is not yet a detailed understanding of the processes determining the delivery, clearance, and accumulation of metals in specific tissues, or of the molecular mechanisms potentially involved in metal-induced malignant transformation or metal effects on tumor behavior.

The current study was designed as a survey of the metal composition of pancreatic juice in the spectrum

Table 3. Comparison of the adjusted mean metal concentrations in pancreatic juice in μg/L, controlling for age, sex, and smoking history, for 35 patients with pancreatic cancer (PC), 30 patients with pancreatitis (P), and 35 patients with normal pancreas (NP)

	PC	P	NP	Pooled	PC vs P	PC vs NP	PC vs (P + NP)
				SD	*P*	*P*	*P*
Ni	22.0	9.9	11.5	29	0.13	0.24	0.13
Cr	30.3	8.0	7.3	45	0.07	0.10	0.05
Co	0.74	0.46	0.45	0.60	0.08	0.11	0.06
Sr	87	35	62	98	0.05	0.40	0.13
Cd	1.2	1.3	1.2	1.2	0.65	0.83	0.71
Pb	4.1	1.3	4.8	7.0	0.13	0.76	0.54
Al	93	65	123	160	0.52	0.56	0.99
Ti	190	170	150	150	0.61	0.36	0.41
V	1.01	0.83	0.70	0.71	0.34	0.16	0.18
Mn	35	62	29	56	0.08	0.71	0.48
Fe	2,100	1,000	1,100	3,900	0.30	0.37	0.28
Cu	310	170	320	290	0.07	0.89	0.38
Zn	2,300	1,100	1,700	2,500	0.08	0.44	0.16
Se	63	50	43	25	0.07	0.01	0.01
Mo	6.5	2.9	2.5	4.7	0.006	0.006	0.002
Ba	190	160	250	420	0.74	0.69	0.95
Ni, Cr, Co, Sr, Cd, Pb	145	56	87	130	0.01	0.15	0.03
Cr, Se	93	58	50	49	0.01	0.005	0.003

Abbreviations: PC, pancreatic cancer; P, pancreatitis; NP, normal pancreas.

Table 4. Adjusted odds of pancreatic cancer among patients with higher metal concentration in pancreatic juice relative to those with lower metal concentration in pancreatic juice, controlling for age, sex, and smoking history

	Unit of change (μg/L)	Odds ratio (95% CI)	*P*
Ni	29	1.51 (0.72-3.2)	0.28
Cr	14	3.1 (1.23-7.8)	0.02
Co	0.52	1.28 (0.62-2.6)	0.50
Sr	66	1.09 (0.73-1.64)	0.67
Cd	1.1	1.18 (1.56-2.5)	0.66
Pb	11	1.00 (0.29-3.4)	1.00
Al	230	0.27 (0.03-2.4)	0.24
Ti	130	1.18 (0.54-2.6)	0.68
V	0.61	1.26 (0.66-2.4)	0.48
Mn	48	0.21 (0.04-1.19)	0.08
Fe	480	1.70 (0.87-3.3)	0.12
Cu	420	0.93 (0.35-2.5)	0.89
Zn	1,400	1.03 (0.63-1.69)	0.90
Se	13	2.6 (0.94-7.1)	0.07
Mo	2.2	1.76 (0.82-3.8)	0.15
Ba	200	0.86 (0.65-1.14)	0.28
Ni, Cr, Co, Sr, Cd, Pb	79	1.31 (0.74-2.3)	0.36
Cr, Se	20	5.8 (1.55-22)	0.009

NOTE: The case group consisted of 35 patients with pancreatic cancer and the reference group consisted of 35 patients with normal pancreas. The unit of change for calculating the odds ratio was the SD of the concentration in the normal pancreas group.

of patients being investigated for abdominal pain felt to be consistent with a pancreatic source. Intensive investigation including endoscopic retrograde cholangiopancreatography separated this group into those with histologically proven pancreatic cancer, those with chronic pancreatitis, and those with normal pancreas. Although the latter group was not entirely ''normal'' based on their having abdominal discomfort, the extensive series of studies provided strong confidence that they had no intrinsic pancreatic disease and likely had some functional cause of their symptoms, such as irritable bowel syndrome or nonulcer dyspepsia. We could not justify performing gastrointestinal endoscopy and endoscopic retrograde cholangiopancreatography on asymptomatic, healthy subjects to add a fully validated control group to the current study. Indeed, we showed distinctive metal composition of the pancreatic juice in patients with pancreatic cancer relative to the groups with chronic pancreatitis and with normal pancreas. Of note, the pancreatic juice metal composition was quite similar for those patients in the latter two noncancer reference groups.

We observed higher concentrations of chromium, selenium, and molybdenum in pancreatic juice of patients with pancreatic cancer relative to those in patients with chronic pancreatitis or normal pancreas. Elevations of the concentrations of these metals were shown to be associated with increases of 210%, 160%, and 76%, respectively, in the odds of having pancreatic cancer in patients investigated for pancreatic-type pain relative to those with normal pancreas. The sum of the concentrations of the two elements with the strongest associations, chromium and selenium, was shown to be associated with a 480% increase in the odds of having pancreatic cancer relative to the reference group. Chromium is already well recognized as having negative effects as a mutagen that avidly binds to and induces DNA damage (single strand breaks), and has the potential to cause cell transformation (24). This has been most clearly established in lung cancer (20), but has also been reported in nasopharyngeal cancer and can act as an enhancer for the development of UV light–induced skin cancer in experimental animals (25-27). In contrast, selenium is actually an essential trace element and is believed to have many positive effects, at least in the concentrations typically present in normal tissue (28, 29).

The source of theses metals has not been established. Because cigarette smoke is an established risk factor for pancreatic cancer and because cigarette smoke is known to contain hazardous metals like chromium, cadmium, and lead, smoking could be a substantial contributor to the presence of such metals in pancreatic juice. However, of these damaging metals, only chromium was significantly elevated in pancreatic juice of patients with pancreatic cancer. Selenium and molybdenum, representing the other metals that were significantly elevated, are not typically found in cigarette smoke. Smoking has even been suggested to reduce selenium levels because smoking-derived zinc competes for pulmonary selenium uptake (7, 28).

Other clues to the sources of these metals in the pancreatic juice could come from the patterns of metal composition observed. Of interest, there was no correlation between the concentrations of chromium and selenium in individuals, suggesting that these metals likely come from distinct sources. Chromium correlated strongly with zinc and nickel. These correlations were stronger in pancreatic cancer patients than in those with normal pancreas. These three metals are recognized components of cigarette smoke and, indeed, these correlations were higher in the smokers than in the nonsmokers. However, the other metal components of cigarette smoke, such as cobalt, lead, and strontium, did not correlate with the chromium. Of all the metals analyzed, selenium correlated strongly only with molybdenum, and this correlation reached significance only in patients with pancreatic cancer.

The correlation between selenium and molybdenum may stem from the chemical similarities of these elements. For example, in oxygenated solutions, both metals occur as divalent oxyanions in which the element, in a hexavalent oxidation state, is coordinated to four O atoms in tetrahedral geometry. As a result of their chemical similarities, these elements may enter the body together from an as-yet-undetermined source. Alternatively, it is possible that biochemical changes induced by pancreatic cancer affect the concentrations of these elements in similar ways. An intriguing but speculative notion is that enrichment in selenium and molybdenum is a direct consequence of the biochemical response to elevated chromium. Such a connection might occur if cellular mechanisms activated to export excessive chromium from tissues to fluids incidentally export selenium and molybdenum as well.

The simultaneous measurement of multiple metals in pancreatic juice also provides the opportunity to gain insights into possible functional interrelationships. For example, chromium avidly binds to DNA and causes single-strand breaks (7), whereas nickel can induce oxidative stress that depletes glutathione and activates

nuclear factor-κB, and other oxidation-sensitive transcription factors (16). Collectively, these two metals may provide conditions for the selection of cells that could go on to become malignant, having changed their energy metabolism or growth requirements or having become resistant to apoptosis. Other combinations of metals can form complexes (such as cadmium oxide) that can cause tumors in animals exposed to other carcinogens (14).

The current observations of abnormalities of metals in pancreatic juice of pancreatic cancer patients are intriguing and clearly support further study of the potential roles of metals in this disease. It is not likely

Table 5. Among all 100 patients, 35 patients with pancreatic cancer, and 35 patients with normal pancreas

A. Among all 100 patients

	Ni	Cr	Co	Sr	Cd	Pb	Al	Ti	V	Mn	Fe	Cu	Zn	Se	Mo
Cr	0.80														
Co	0.21	0.04													
Sr	0.19	0.21	−0.07												
Cd	0.00	−0.11	0.10	0.25											
Pb	0.01	0.04	0.20	0.14	−0.02										
Al	0.06	0.05	0.13	0.22	0.12	−0.03									
Ti	0.05	0.01	0.23	0.17	−0.05	−0.06	0.28								
V	0.45	0.41	0.07	0.27	0.05	−0.07	−0.05	0.29							
Mn	0.10	0.10	−0.02	0.02	0.15	−0.06	0.13	0.27	0.13						
Fe	0.02	0.04	0.27	0.05	0.14	0.06	0.07	0.23	−0.05	−0.03					
Cu	0.14	0.16	0.01	0.48	0.08	0.21	0.25	0.24	0.16	0.07	0.06				
Zn	0.42	0.63	0.22	0.26	0.11	0.12	0.10	0.19	0.35	0.03	0.59	0.22			
Se	0.10	−0.02	0.29	0.09	0.34	0.13	0.11	0.20	0.05	−0.03	0.22	0.00	0.16		
Mo	0.22	0.11	−0.07	0.14	0.16	0.04	−0.05	0.14	0.26	−0.04	−0.03	0.04	0.08	0.70	
Ba	−0.08	−0.07	0.49	0.11	0.20	0.17	−0.03	−0.05	0.19	−0.09	−0.02	0.08	0.16	0.27	0.08

B. Among the subgroup of 35 patients with pancreatic cancer

	Ni	Cr	Co	Sr	Cd	Pb	Al	Ti	V	Mn	Fe	Cu	Zn	Se	Mo
Cr	0.92*														
Co	−0.13*	−0.16*													
Sr	0.24	0.20	−0.21												
Cd	−0.20	−0.24	0.08	0.43											
Pb	−0.00	0.03	0.08	0.41	0.21										
Al	0.15	0.20	0.21	0.09*	0.04	−0.04									
Ti	0.06	−0.01	0.45*	0.05	0.04	0.14	0.32								
V	0.55	0.59	0.04	0.36	0.18	0.11	0.08	0.18							
Mn	0.35	0.41*	−0.05	0.30	0.26	0.01	0.34	0.26	0.58						
Fe	−0.06	−0.02	0.23	0.01	0.08	0.22	0.20	0.36*	−0.11	0.03					
Cu	0.33	0.31	−0.07	0.75*	0.14	0.37	0.26	0.39	0.47*	0.39	0.11				
Zn	0.57*	0.67*	0.15	0.24	−0.05*	0.29	0.25	0.32*	0.51*	0.35	0.62*	0.41			
Se	0.01	−0.13	0.21	0.02	0.38	0.10	0.10	0.28	0.15	−0.10	0.16	−0.03	0.09		
Mo	0.26	0.07	−0.17	0.09	0.21	0.11	−0.15	0.08	0.30	−0.10	−0.08	0.07	0.03	0.81 †	
Ba	−0.12	−0.10	0.58	0.06	0.28	0.16	−0.05	0.01	0.32	−0.12	−0.04	0.00	0.11	0.31	0.05

C. Among the subgroup of 35 patients with normal pancreas

	Ni	Cr	Co	Sr	Cd	Pb	Al	Ti	V	Mn	Fe	Cu	Zn	Se	Mo
Cr	0.78 ‡														
Co	0.54 ‡	0.55 ‡													
Sr	0.02	−0.06	−0.02												
Cd	0.11	0.06	0.07	0.02											
Pb	−0.01	0.09	0.34	0.05 ‡	−0.12										
Al	0.05	−0.11	0.06 ‡	0.56 ‡	0.19	−0.07									
Ti	−0.05	−0.20	−0.31 ‡	0.44	−0.03	−0.19	0.45								
V	0.66	0.32 ‡	0.08	0.02	0.19	−0.17	−0.12	0.23							
Mn	−0.06	−0.10 ‡	−0.29	0.01	0.51	−0.09	−0.09	0.18 ‡	0.18						
Fe	0.05	0.07	0.49	−0.15 ‡	0.30	−0.12	−0.08	−0.15 ‡	0.01 ‡	−0.03					
Cu	0.00 ‡	−0.02 ‡	−0.07	0.33 ‡	0.08 ‡	0.16	0.21	0.08 ‡	−0.06 ‡	−0.06	−0.01 ‡				
Zn	−0.01 ‡	0.09 ‡	0.17	0.06	0.51 ‡	0.08	−0.05	−0.22 ‡	0.04 ‡	−0.10	0.16 ‡	−0.03			
Se	0.11	0.06	0.22	0.15	0.08	0.38	0.19	0.13	0.05	0.00	−0.22	−0.02	0.22		
Mo	0.08	0.13	−0.15	0.20	0.13	−0.03	0.00	0.39	0.09	0.14	−0.10	−0.07	0.06	0.28§	
Ba	−0.12	−0.01	0.36	0.35	0.25	0.41	−0.11	−0.36	−0.15	−0.07	0.29	0.31	0.48	0.20	0.11

*$P < 0.05$, versus patients with normal pancreas.
† $P < 0.001$, versus patients with normal pancreas.
‡ $P < 0.05$, versus patients with pancreatic cancer.
§ $P < 0.001$, versus patients with pancreatic cancer.

Table 6. Correlations between concentrations of metals in pancreatic juice among current smokers and patients who never smoked

A. Among the subgroup of 24 patients who were current smokers

	Ni	Cr	Co	Sr	Cd	Pb	Al	Ti	V	Mn	Fe	Cu	Zn	Se	Mo
Cr	0.96*														
Co	−0.16 †	−0.14													
Sr	0.41	0.34	−0.13												
Cd	−0.06	−0.18	0.04	0.40											
Pb	0.04	0.03	0.35	0.03	−0.07										
Al	0.18	0.06	0.20	0.24	0.30	−0.08									
Ti	0.12	0.05	0.27	0.19	−0.03	−0.21	0.50								
V	0.77 ‡	0.77*	−0.12 ‡	0.37	−0.18	−0.11	−0.03	0.33							
Mn	0.13	0.16	0.36	0.20	0.09	−0.09	0.28	0.44	0.12						
Fe	0.15	0.15	0.23	0.15	0.69 ‡	−0.06	0.04	−0.09	0.07	0.14					
Cu	0.26	0.23	−0.02	0.28 ‡	0.20	0.14	0.19	0.08	0.15	0.15	0.07				
Zn	0.94*	0.97*	−0.06	0.37	−0.11 ‡	0.06	0.10	0.04	0.76 ‡	0.26	0.19	0.27			
Se	0.11	−0.07	0.00 ‡	−0.03	0.40	0.10	0.02	0.10	−0.09	−0.01	0.16	−0.10	−0.08 ‡		
Mo	0.30	0.11	−0.23 ‡	0.00	0.18	−0.02	−0.12	0.07	0.22	−0.16	−0.02	−0.10	0.06	0.85 ‡	
Ba	−0.13	−0.12	0.58	−0.23 ‡	−0.12	0.49	−0.10	−0.17	−0.14 ‡	0.06	−0.14	0.29	−0.04 ‡	−0.08 ‡	−0.07

B. Correlations between concentrations of metals in pancreatic juice among the subgroup of 49 patients who never smoked

	Ni	Cr	Co	Sr	Cd	Pb	Al	Ti	V	Mn	Fe	Cu	Zn	Se	Mo
Cr	0.78 ‡														
Co	0.40§	0.31													
Sr	−0.03	−0.09	0.14												
Cd	0.06	−0.02	0.23	0.03											
Pb	−0.10	−0.13	0.15	0.36	−0.03										
Al	−0.14	−0.16	0.08	0.37	−0.05	−0.01									
Ti	0.08	0.04	0.35	0.37	−0.04	0.08	0.35								
V	0.33§	−0.06 ‡	0.45§	0.13	0.13	0.02	−0.04	0.20							
Mn	0.14	0.19	−0.01	−0.03	0.07	0.02	0.11	0.26	0.10						
Fe	0.06	0.08	0.14	−0.01	0.20§	−0.09	0.14	−0.04	−0.04	−0.02					
Cu	−0.05	−0.08	0.14	0.76§	−0.07	0.42	0.45	0.38	0.09	0.08	−0.05				
Zn	0.01 ‡	−0.00 ‡	0.40	0.13	0.60§	0.14	0.01	0.05	0.20§	−0.06	0.17	0.04			
Se	0.06	−0.01	0.67§	0.18	0.46	0.03	0.15	0.40	0.28	0.02	0.34	0.00	0.45§		
Mo	0.13	0.10	0.32§	0.29	0.22	0.11	0.14	0.46	0.38	0.12	0.02	0.01	0.25	0.40§	
Ba	−0.11	−0.14	0.59	0.31§	0.32	0.28	−0.03	0.00	0.36§	−0.12	0.01	0.10	0.45§	0.57§	0.34

*$P < 0.001$, versus never smoked.
†$P < 0.05$, versus never smoked.
‡$P < 0.001$, versus current smokers.
§$P < 0.05$, versus current smokers.

that determination of the concentrations of metals in pancreatic juice will become a clinical test, due to the invasive nature of the collection of this fluid that is necessary. In the future, it will be important to understand the metal composition of the histologically normal pancreas and pancreatic cancer tissue, as well as that of other body fluids in patients with pancreatic cancer. With the new insights into the concentrations of metals that are present in pancreatic juice, it will also be critical to study the effects of these individually and together on pancreatic cell lines.

Acknowledgments

We thank the W.M. Keck Foundation Laboratory for Environmental Biogeochemistry at Arizona State University, where inductively coupled plasma mass spectrometric analyses were conducted, and Brie N. Noble for help with statistical analysis.

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