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Differences in K-ras and p53 gene mutations among pancreatic adenocarcinomas associated with regional environmental pollution

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***Background*: Variations in genetic mutations in pancreatic carcinoma between different geographical regions have not been studied extensively, especially in developing countries where pancreatic cancer is relatively rare. *Methods*: We studied the molecular pathology of 54 pancreatic adenocarcinomas from Egyptian patients residing in a heavily polluted region of the eastern Nile River delta and compared the findings with 45 tumors from patients residing in low-pollution regions. *Results*: Rates of *K-ras* mutation in codon 12 and of *p53* mutation in exons 5–8 were higher in tumors of patients from the high-pollution region as compared with the low-pollution regions (61.5 versus 34.2%, respectively, for *K-ras*, $P = 0.01$; 25.9 versus 11.6%, respectively, for *p53*, $P = 0.08$). There were also distinct differences in the specific types of *K-ras* and *p53* mutations between the two regions. The ratio of G-to-T *k-ras* transversion mutation (codon 12) relative to wild-type was significantly higher in tumors from the high-pollution region (0.90) than tumors from the non-pollution site (0.28) ($P = 0.03$). Relative to tumors with wild-type, the ratio of *p53* mutations in exons 5, 7 or 8 to wild-type in tumors from the high-pollution region was significantly higher than the ratio from the non-pollution site (0.28 versus 0.03, $P = 0.01$). Logistic regression showed that G-to-T transversion mutation in *K-ras* was predicted by the region of residence of the patients. *Conclusions*: Our study reveals that there are differences in the frequencies and types of *K-ras* and *p53* mutations found in pancreatic adenocarcinomas of patients in high-pollution and low-pollution regions in Egypt and suggests that environmental factors may explain these differences. We speculate that gene–environment interactions in pancreatic carcinogenesis also occur in other populations.**

Introduction

Little is known about the etiology of pancreatic cancer, although this tumor is the most lethal of the major malignancies. The molecular genetics of pancreatic adenocarcinoma have been studied in detail, and mutations in the *K-ras* proto-oncogene and *p53* gene are the most common DNA sequence alterations (1). Mutations in other genes such as smad4, p16 and Gadd45 have also been identified in pancreatic carcinogenesis (2).

Studies of unusual cancer distribution and analysis of particular genetic mutations may provide clues to cancer etiology. International variations in frequencies and types of mutations in pancreatic tumors have been reported. For example, variable rates of G-to-T and G-to-A mutations were described in patients from different populations (3–6). Furthermore, associations between mutation types and certain environmental exposures have been reported. Alguacil *et al.* (7) analyzed the occupational exposures of 107 incident pancreatic cancer patients from Spain and reported an association between chromium exposure and G-to-T transversions in *K-ras*. Other studies relating environmental pollution and genetic mutations in pancreatic cancer have also been published (8,9).

We recently reported patterns of genetic mutations in Egyptian pancreatic adenocarcinomas that were distinct from those in USA (6). Tumors included in our previous study were from patients residing in a heavily polluted region in the eastern Nile River delta around Mansoura (10–13). The soil of the polluted region where our previous research was done has been analyzed extensively and shown to have the highest heavy metal and pesticide levels in Egypt, with steadily decreasing levels of these pollutants upstream in the Nile River basin in a southerly direction (14,15). The study reported here was designed to evaluate the rates and types of *K-ras* and *p53* mutations in tumors from Egyptian patients who resided in different geographical regions of the country with a wide range of environmental pollution. The study also compared and contrasted the mutation characteristics from these regions.

Materials and methods

Sources of patients and data collected

This study included 99 histologically confirmed ductal adenocarcinomas of the pancreas obtained from newly diagnosed patients who underwent surgical resection of stage II and III pancreatic adenocarcinomas (16) in Egypt. Fifty-four tumors were from the Gastrointestinal Surgery Center of Mansoura University in the heavily polluted eastern Nile River delta region, 44 of these tumors were included in our previous publication (6); 27 tumors were from the National Cancer Institute of Cairo University; 9 tumors from Tanta Cancer Center in the center of the Nile delta region and 9 tumors from the South Valley Cancer Center of Assiut University in South Egypt. All cases represented consecutive patients who underwent resection of the pancreas after a preliminary histopathological diagnosis of pancreatic adenocarcinoma. For our study, the diagnosis was confirmed by at least one senior pathologist in each Egyptian cancer hospital, followed by centralized review and confirmation at the University of Texas M. D. Anderson Cancer Center in Houston by one of the authors (S.R.H.).

All patients were residents of the provinces where the hospitals are located, and no age or gender restrictions were applied. The patients from all hospitals were consecutive patients who had a surgical resection for pancreatic cancer during the period of the study with recruitment from November 1998 to February 2004. Those patients represented ~26% of all incident pancreatic cancer patients seen at the study hospitals during this period. The patients from the hospitals in Mansoura and Cairo were participants in ongoing studies to investigate the molecular epidemiology of pancreatic cancer in the two institutions. The tumors from Tanta and Assiut were obtained as archival tissue from the surgical pathology files of patients who underwent diagnosis and treatment by surgical resection for adenocarcinoma of the pancreas during the same time period. There was no significant difference in the stage of tumors between the two groups from Mansoura and non-Mansoura. All tumors included in this study from the two sites were stages II and III.

Interviewers at Mansoura and Cairo elicited epidemiologic information using a questionnaire that had been used and tested in previous studies (17–19). The questionnaire allowed collection of information about demographics, occupation (agricultural, professional and technical/administrative), residence (urban versus rural), smoking and any family history of cancer. Interviews of patients took place during the routine 3- to 7-day hospital admission for their pre-operative clinical and laboratory evaluations. Trained interviewers who participated in our previous studies (13,19,20) conducted the interviews. The medical records of the patients from Tanta and Assiut were reviewed to retrieve the same data elements. A trained study coordinator from National Cancer Institute of Cairo University abstracted the medical records under the supervision of three of the co-authors (A.S.S., I.A.S. and A.A.). The study was approved by Institutional Review Board (IRB) committees in Egypt and the USA.

Laboratory methods

Microdissection and DNA extraction. Areas of adenocarcinoma and non-neoplastic control tissue were microdissected from routine formalin-fixed, paraffin-embedded tissue sections cut 5 μm thick and stained with hematoxylin and eosin. DNA was extracted as in our previous studies (6,13). Approximately one square centimeter of tumor tissue was wet with xylene, scraped from the slide using a clean razor blade and placed into a microcentrifuge tube. The xylene was removed by vacuum centrifugation until the specimen was completely dry. Each specimen was then treated with 50 μl of buffer containing 0.5% Tween 20 (Boehringer Mannheim, Mannheim, Germany), 20 μg proteinase K (Boehringer Mannheim), 50 mM Trizma Base at pH 8.9 and 2 mM ethylenediaminetetraacetic acid. The samples were incubated at 56°C overnight. Proteinase K was inactivated by incubating the samples at 100°C for 10 min. A 1:20 dilution of the DNA lysate in nuclease-free water was used for polymerase chain reaction (PCR) amplification. The extracted DNA was stored at −20°C until analysis.

K-ras PCR amplification. We evaluated codons 12 and 13 of the *K-ras* gene, and the mutations were classified for location in either the first or the second base (21). PCR amplification was done in a 50 μl reaction volume using 2 μl of genomic DNA, 1× PCR Buffer II, 2 mM magnesium chloride, 0.8 mM deoxynucleoside triphosphate mix, 2.25 U AmpliTaq™ Gold (Applied Biosystems, Foster City, CA), 0.125 U Pfu DNA polymerase (Stratagene, La Jolla, CA) and 20 pmol of each primer (forward primer, 5′-GGCCGGTAGTGTATTAACCTTATGTGTGACAT-3′ and reverse primer, 5′-CCGCGGCCGGCGGCCAAAACAAGATTTACCTCTATTGTTGG-3′; Life Technologies, Gaithersburg, MD). PCRs were carried out using the following touchdown cycling conditions: denaturation at 95°C for 10 min; 14 cycles [95°C × 20 s, 59°C × 60 s (−0.5°C per cycle) and 72°C × 60 s]; 25 cycles (95°C × 20 s, 52°C × 60 s and 72°C × 60 s) and extension at 72°C for 10 min. Thermal cycling was performed using a GeneAmp PCR System 9700 (Applied Biosystems). The quality of the product was analyzed on a 6% polyacrylamide gel.

p53 PCR amplification. Exons 5 through 8 of the *p53* gene were amplified separately in 50 μl volumes using ∼2 μl of the 1:20 diluted DNA lysate, 1× PCR Buffer II (Applied Biosystems), an additional 100 nmol magnesium chloride, 40 nmol deoxynucleoside triphosphate mix, 1.25 U AmpliTaq™ Gold (Applied Biosystems) and 20 pmol of sense and anti-sense primer. The primer sequences were as follows: exon 5 (sense) 5′-GACTTTCAACTCTGTCTCC-3′ and exon 5 (anti-sense) 5′-GAGCAATCAGTGAGGAATC-3′, exon 6 (sense) 5′-TCCCCAGGCCTCTGATTCC-3′ and exon 6 (anti-sense) 5′-TGACAACCACCCTTAACCC-3′, exon 7 (sense) 5′-CAAGGCGCACTGGCCTCATC-3′ and exon 7 (anti-sense) 5′-CACAGCAGGCCAGTGTGCAG-3′ and exon 8 (sense) 5′-GATTTCCTTACTGCCTCTTGC-3′ and exon 8 (anti-sense) 5′-GTGAATCTGAGGCATAACTGC-3′.

PCR amplification was carried out using the following cycling conditions: for exons 6 and 8, denaturation at 95°C for 10 min, 45 cycles (95°C × 60 s, 61°C × 60 s and 72°C × 60 s) and extension at 72°C for 5 min; for exon 7, denaturation at 95°C for 10 min, 45 cycles (95°C × 60 s, 65°C × 60 s and 72°C × 60 s) and extension at 72°C for 5 min and for exon 5, denaturation at 95°C for 10 min, 45 cycles (95°C × 60 s, 55°C × 60 s and 72°C × 60 s) and extension at 72°C for 5 min. Thermal cycling was performed using a GeneAmp® PCR System 9700 (Applied Biosystems). The quality of the product was examined and quantitated on a 2.0% agarose gel.

Automated DNA sequencing for K-ras and p53 mutations. PCR products were diluted to 5 ng/μl and purified by mixing 5 μl of PCR product dilution with 2 μl Exo/SAP (Amersham Life Science, Cleveland, OH), incubated at 37°C for 15 min and then inactivated by incubating at 80°C for 15 min. DNA sequencing was performed in 20 μl volumes using 2 μl purified PCR product, 4 μl ABI PRISM® BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems) and 10 pmol of forward primer using the following cycling conditions: initial denaturation at 95°C × 5 min followed by 25 cycles of 95°C × 20 s, 52°C × 60 s and 60°C × 60 s. After spin column purification (Princeton Separations, Freehold, NJ) and re-suspension in 10 μl formamide, the reaction products were sequenced by capillary electrophoresis using an ABI PRISM™ 3700 or ABI 3730 DNA Analyzer (Applied Biosystems). The sequence of each sample containing mutation was confirmed using the reverse primer.

K-ras amplification and sequencing was successful in 52/54 (96.3%) of tumors from Mansoura and in 38/45 (84.4%) of tumors from the other group ($P = 0.24$). *p53* sequencing was successful in all tumors from Mansoura and in 43/45 (95.6%) of tumors from the other group ($P = 0.20$, Table I).

Statistical methods and analysis

Because of the relatively small number of specimens included in the study from Tanta Cancer Center, National Cancer Institute of Cairo University and Assiut Cancer Center and the geographical location of these institutions in low-pollution regions as contrasted with the high-pollution region of Mansoura, we grouped the tumors into Mansoura and non-Mansoura groups for analysis. Differences in frequencies between the two groups were evaluated by simple contingency table analysis (Fisher's exact probability test) using the SAS® statistical package (SAS® v8.2). Variables that were included in this analysis were age (used as a continuous variable), gender (male versus female), smoking (yes versus no) and residence for Egyptians (rural versus urban). Residence data for patients reflected lifetime residence. Occupation was classified into two groups (agricultural versus others) and family history of pancreatic cancer and family history of cancer in any relative were categorized as yes versus no. Total frequencies of *K-ras* and *p53* mutations were used in the analysis as categorical variables, along with single-nucleotide changes and codon/exon position.

Unconditional logistic regression models were computed to test associations between mutation types and demographic, occupational and lifestyle factors. Contingency tables were constructed yielding chi-squared *P*-values, Fisher's exact *P*-values, crude odds ratio (OR) and 95% confidence interval (95% CI). Separate multiple logistic regression models were constructed for statistical analysis of *K-ras* G-to-T and G-to-A mutation and *p53* mutation in exon 6 as the dependent variables with other variables (e.g. residence, gender, smoking and farming-related occupations) as the covariates to test for confounding and effect modification. *K-ras* mutations were included in the logistic regression model as the ratio of the number of tumors with specific mutations, e.g. G-to-T or G-to-A, to the number with a wild-type tumor (7). Because of the small sample size of tumors with mutation in exons 7 and 8 of *p53*, it was not possible to perform the logistic regression analysis for these mutations.

Table I. Characteristics of pancreatic cancer patients from the heavily polluted Mansoura region and the less-polluted non-Mansoura region in Egypt

	Mansoura ($N = 54$) No. (%)	Non-Mansoura[a] ($N = 45$) No. (%)	*P*-value
Age			
Mean (SD)	53.8 (10.4)	55.1 (11.6)	0.56[b]
Gender			
Male	35 (64.8)	23 (51.1)	
Female	19 (35.2)	22 (48.9)	0.17[c]
Residence			
Rural	35 (64.8)	19 (45.2)	
Urban	19 (35.2)	26 (57.8)	0.03[c]
Occupation			
Agriculture	36 (66.7)	14 (31.1)	
Others	18 (33.3)	31 (68.9)	0.001[c]
Smoking			
Yes	30 (55.6)	17 (37.8)	
No	24 (44.4)	28 (62.2)	0.08[c]
Family history of any cancer			
Yes	5 (9.3)	1 (2.2)	
No	49 (90.7)	44 (97.8)	0.22[d]
K-ras codon12[e] (52,38)			
Mutation	32 (61.5)	13 (34.2)	
Wild-type	20 (38.5)	25 (65.8)	0.01[c]
p53 × 5, 6, 7, 8[f] (54,43)			
Mutation	14 (25.9)	5 (11.6)	
Wild-type	40 (74.1)	38 (88.4)	0.08[c]
K-ras and *p53* mutation[g] (32,12)			
	9/32 (28.1)	3/12 (25.0)	1.00[c]

[a]Non-Mansoura area included 27 subjects from Cairo and 9 subjects each from Tanta and Assiut.
[b]*t*-test.
[c]Fisher's exact test.
[d]Chi-square.
[e]Two patients from the Non-Mansoura region with *K-ras* mutation in codon 13 were excluded from analysis of genetic types
[e,f]Sample size varied due to missing data.
[g]The denominator of 32 and 12 represent tumors with *K-ras* mutations in the two groups. One tumor with failed *p53* results was excluded from the nominator and denominator of non-Masoura group.

Results

Patient characteristics

The two groups of patients from the high-pollution and low-pollution areas included in this study did not have significant differences in age

or gender (Table I). Mean age of patients from Mansoura was 53.8 ± 10.4 versus 55.1 ± 11.6 years for the non-Mansoura group from the other three centers ($P = 0.56$). Patients from Mansoura included 35 (64.8%) males and 19 (35.2%) females whereas the patients from the other group included 23 males (51.1%) and 22 (48.9%) females ($P = 0.17$, Table I). Patients from Mansoura were significantly different from patients in the other group with respect to place of residence and occupation (Table I). Significantly, more patients from Mansoura had rural residence (rural:urban distribution of 64.8 and 35.2% as compared with 45.2 and 57.8%, $P = 0.03$). In addition, patients from Mansoura more frequently held agricultural jobs (66.7%) compared with 31.1% with agricultural jobs in the other group ($P = 0.001$). More patients were smokers among the patients from Mansoura (55.6 versus 37.8%, $P = 0.08$) and more patients had family history of cancer among the Mansoura patients (9.3 versus 2.2%, $P = 0.22$), but these differences were not statistically significant.

Mutation frequencies

Overall, the rates of *K-ras* were statistically different in tumors of patients from Mansoura and the non-Mansoura group (61.5 versus 34.2%, $P = 0.01$, Table I). Rates of *p53* mutations were not statistically different (25.9% in Mansoura versus 11.6%, $P = 0.08$, Table I). The frequency of tumors with both *K-ras* and *p53* mutations was similar in the two groups (28.1% compared with 25.0%, $P = 1.00$, Table I).

Types of mutations

The ratios of individual types of mutations of *K-ras* and *p53* to wild-type were different between the two groups of tumors. The ratio of G-to-T *K-ras* mutations to wild-type was significantly higher in tumors from Mansoura (0.90) than non-Mansoura (0.28) ($P = 0.03$) (Table II). The ratio of G-to-A transitions to wild-type was also higher in tumors from Mansoura than tumors from non-Mansoura (0.70 versus 0.24 in the two groups, respectively). *K-ras* G-to-C transversions were not detected in any of the tumors in this study.

Individual types of *p53* mutations did not differ significantly between the two groups of tumors (Table II). However, relative to tumors with wild-type, the ratio of *p53* mutations in exons 5, 7 or 8 to wild-type in tumors from the high-pollution region was significantly higher than the ratio from the non-pollution site (0.28 versus 0.03, $P = 0.01$).

Logistic regression analysis showed that place of residence of patients (Mansoura versus non-Mansoura) was an important predictor of *K-ras* G-to-T mutations ($P = 0.03$, Table III).

After adjustment for age, gender, smoking and occupation as potential confounders, place of residence was strongly and independently predictive of *K-ras* G-to-T transversion ($P = 0.03$), and no evidence of confounding was identified when tested against other confounders (Table III). We found that tumors from Mansoura were 3.6 times more likely to have this mutation than others (95% confidence interval: 1.1–11.3).

Place of residence of patients was not an important predictor of *K-ras* G-to-A mutation in models that adjusted for age, gender, smoking and occupation (Table IV). Odds ratio of place of residence was OR = 3.1 (95% CI: 0.9–11.4, $P = 0.09$) for the adjusted model that included age, gender and occupation and rural/urban residence. *p53* was not included in logistic regression analysis because of the small sample size of individual mutations.

Table II. Distribution of *K-ras* mutation in codon 12 and of *p53* mutation in exons 5–8

	Mansoura		Non-Mansoura		*P*-value[a]
	No.	Ratio of mutant to wild-type	No.	Ratio of mutant to wild-type	
Type of K-ras mutation in codon 12					
G-to-T	18	0.90	7	0.28	0.03
G-to-A	14	0.70	6	0.24	0.07
G-to-C	0	0	0	0	N/A
Wild-type	20		25		
Type of p53 mutation					
Exon 6	3	0.08	4	0.11	0.71
Exon 5, 7 or 8	11	0.28	1	0.03	0.01
Exon 5	6	0.15	1	0.03	0.12
Exon 7	3	0.08	0	0	0.25
Exon 8	2	0.05	0	0	0.49
Wild-type	40		38		

N/A, Not applied.
[a]Fisher's test.

Table III. Logistic regression model to predict G-to-T transversions in *K-ras* gene

	Odds ratio	95% confidence interval	*P*-value
Unadjusted geographic area			
Non-Mansoura	1		
Mansoura	3.21	1.12–9.21	0.03
Adjusted geographic area			
Non-Mansoura	1		
Mansoura	3.58	1.13–11.29	0.03
Age			
≤50	1		
>50	1.02	0.34–3.06	0.98
Gender			
Female	1		
Male	0.74	0.20–2.81	0.66
Occupation			
Other	1		
Agriculture	0.57	0.09–3.80	0.57
Residence			
Urban	1		
Rural	1.57	0.26–9.39	0.62
Smoking			
No	1		
Yes	0.99	0.28–3.52	0.99

Table IV. Logistic regression model to predict G-to-A transitions in *K-ras* gene

	Odds ratio	95% confidence interval	*P*-value
Unadjusted geographic area			
Non-Mansoura	1		
Mansoura	2.92	0.95–8.96	0.06
Adjusted geographic area			
Non-Mansoura	1		
Mansoura	3.12	0.85–11.39	0.09
Age			
≤50	1		
>50	2.16	0.58–7.97	0.25
Gender			
Female	1		
Male	1.06	0.25–4.53	0.93
Occupation			
Other	1		
Agriculture	1.05	0.18–6.00	0.96
Residence			
Urban	1		
Rural	1.03	0.19–5.45	0.97
Smoking			
No	1		
Yes	1.33	0.33–5.32	0.69

Discussion

The rates of mutation in the *K-ras* proto-oncogene in the two groups of Egyptian pancreatic adenocarcinomas included in this study showed statistically significant differences. In addition, there was a trend toward differences in *p53* mutation rates that did not reach statistical significance. The individual types of mutations showed distinct differences between patients from the two groups within Egypt. The overall rates of *K-ras* and *p53* mutations observed in this study for tumors from Mansoura were comparable with our previous study from the same region, whereas the tumor mutation rates for the non-Mansoura regions were comparable with rates from our previous study of USA patients and to other studies from Western countries (3–5,22–24).

The prevalence of *K-ras* mutations in pancreatic cancer has been reported in many studies with a wide range of frequencies (25). Previous studies comparing the prevalence of *K-ras* mutations in tumors from African-American and Caucasian pancreatic cancer patients in Detroit showed *K-ras* mutations in 70% of African-Americans and 73% of Caucasians (26). Other studies showed *K-ras* mutations in 62, 71 and 75% of subjects from Japan (27), China (28) and Austria (3), respectively. *p53* mutation has been reported with rates of 50–75% in different studies (29). Rates similar to ours have been reported in other studies, i.e. 38% (27) and 41% (23).

The rates of specific mutations in *K-ras* and *p53* in the tumors from the two regions in Egypt showed distinct differences in our study. *K-ras* G-to-T mutations were significantly more frequent in tumors from Mansoura patients than in patients from other Egyptian regions and G-to-C mutations were not identified in any tumors from Egyptian patients in this or previous studies. Furthermore, exon 5 mutations in *p53* were more frequent in tumors from Mansoura patients, whereas exon 6 mutations were more frequent in tumors from the non-Mansoura patients. Rates of types of *K-ras* and *p53* mutations in the non-Mansoura patients in this study were comparable with previous studies from Western countries (30). The distinct mutational types in this study (*K-ras* G-to-T mutations) were predicted by the region of patient's residence (Mansoura or the non-Mansoura) after adjustment for age, gender, smoking and occupation.

A higher proportion of Mansoura patients were farmers and rural residents, with presumed exposure to organochlorine pesticides (17,18). Those compounds have been associated with *K-ras* G-to-T mutations in pancreatic cancer in a previous study in Spain (8). Other more prevalent environmental exposures in Mansoura than the non-Mansoura regions include heavy metals such as cadmium and chromium (10,14,15,31,32). Our recent study in Mansoura revealed significantly higher serum cadmium levels in pancreatic cancer patients than controls (13).

The northeastern part of the Nile River water near Mansoura is heavily polluted with heavy metals, pesticides and hydrocarbons because of increasing discharge of untreated industrial by-products and wastes, other unrecyclable wastes and agricultural irrigation wastewater into the Nile River in that area (33). Sewage from Cairo and five provinces in the delta is also dumped into the Manzala lagoon in the northeast Nile delta region, the most polluted area of the country. The discharge of sewage and industrial wastes into the lagoon increases its contamination levels from heavy-metal and high-nutrient run-off as well as discharge from municipal sewage, industrial effluent and agricultural run-off (34,35). Cadmium concentrates in fish (36) and its concentration was found in very high levels in fish and geochemical features of sea and lake soil (37).

The Smithsonian Institution in Washington, DC, conducted a 10-year study investigating the soil sediments, pollution and heavy metals in the northeastern Nile delta and other parts in Egypt (10). Several reports on Northeast Nile delta region 'Dakahleya Province' and its capital Mansoura and its regional lagoon 'Manzala lagoon' documented the high levels of heavy metals and organochlorine pesticides in the soil and water of the region (10,14,15,31,32, 38). Other studies have also documented the significant difference in pollution levels in Egypt, with highest levels in the Northeast region (Mansoura) and a gradual decrease in pollution in a southern direction (10,14, 15,31,32,38–40). Not only were high levels of cadmium found in the soil in the northeast Nile delta region but also in the serum of fishermen and other apparently healthy residents of the region (41). These serum levels in the population of the northeast Nile delta region were significantly higher than the levels in populations in Cairo (41–43).

Alguacil *et al.* (7) reported that the association between chromium exposure and *K-ras* mutations was statistically significant for G-to-T transversions. Hence, organochlorine pesticides and heavy metals may have contributed to the types of mutations seen in tumors from Egyptian patients at significantly higher rates in Mansoura tumors than those from the non-Mansoura regions.

Chromium III, as well as chromium IV compounds showed stronger tendency to induce G-to-T transversions, as shown in several experimental studies on the mutational spectrum of chromium. Chromium III induced base-substitution hot spots that were different from those occurring spontaneously in human embryonic kidney cell lines (44). Chromium IV in SV40-immortalized human fibroblasts resulted in G-to-T, as the highest rate of mutations (45,46). Nickle, a toxic, mutagenic and carcinogenic metal resulted in G-to-T transversions in codon 12 of the *K-ras* gene in rat renal sarcoma induced with nickle subsulfide (47). *K-ras* G-to-T mutations may reflect the action of some carcinogens prompted by organochlorine pesticide analogs, such as benzo[*a*]pyrene, which experimental models linked it to G-to-T mutations (48,49).

Subjects included in this study had high rates of cigarette smoking with higher, although not statistically significant, rates in the Mansoura patients than in non-Mansoura patients. Smoking has been linked to pancreatic cancer in many studies and is the most consistent risk factor for this disease. However, smoking was not related to specific *K-ras* or *p53* mutations in our study. Although this study included information about cigarette smoking of participants, other sources of smoking such as passive smoking, water pipes and cigar smoking were also associated with pancreatic cancer risk in Egypt (50). These findings support our implication of environmental pollution in producing the genetic differences we observed.

Our study has strengths that support the validity of the findings. First, the histopathological diagnosis of ductal adenocarcinoma of the pancreas and confirmation of the diagnoses by two panels of pathologists in Egypt and the USA is an advantage. Second, the comparability of age, gender and tumor stage minimized the bias of comparison between tumors included in the study. Third, the heavily polluted locale from which patients were recruited in Mansoura and the relatively low-polluted locales in the other regions in Egypt presented an ideal situation to examine the differences in types and rates of mutations between the two regions. It is important to note that there are no ethnic differences between populations in Mansoura and non-Mansoura areas. Ethnic groups in Egypt are the following: Egyptian 98%; Berber, Nubian, Bedouin and Beja 1% and Greek, Armenian and other European (primarily Italian and French) 1% (51). All the patients included in our study were Egyptians.

It is interesting to note that the results of this study showed lower *K-ras* mutation rates in tumors from non-Mansoura patients than tumors of patients from Mansoura. Our previous studies from Egypt comparing colorectal cancers from the non-Mansoura regions in Egypt and the USA showed much lower rates of *K-ras* mutations in Egypt than the USA (11 versus 67%, respectively) (20). Moreover, the types of *K-ras* mutations were significantly different in the pancreatic tumors from the two countries (6). The *K-ras* gene may play a different role in the molecular pathway in different cancers. *K-ras* mutation is an early event in pancreatic cancer (52), and the majority of pancreatic cancers should have *K-ras* mutations, but the time at which the mutation occurs may be induced by different environmental exposures (8,53–56).

In summary, pancreatic cancers from Egyptian patients residing in polluted regions in this study had unique patterns of *K-ras* and *p53* mutations that were significantly different from the patterns of mutations detected in tumors from Egyptian patients who resided in

non-polluted regions. The differences in *K-ras* and *p53* mutations may be associated with high levels of environmental exposures, such as organochlorine pesticides or heavy metals, especially in the heavily polluted region where the Egyptian patients were recruited (13). Future studies should compare the variety of mutations for patients in different regions in Egypt and the USA with different pollution levels. Detailed information about different types of smoking should also be included in future studies. Such studies may provide more evidence to support the association between mutational types and environmental and lifestyle exposures. Determining the level of cadmium and other heavy metals in blood or tissue of pancreatic cancer patients, and examining their association with mutational types, may provide clues about the etiology of pancreatic cancer and the role of gene–environmental interactions in pancreatic carcinogenesis.

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