

Short Communication

Selective Expression of CYP2A13 in Human Pancreatic α -Islet Cells

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ABSTRACT:

Exposure to cigarette smoke is an etiological factor of human pancreatic cancer and has been associated with an increased risk of pancreatic diseases, including pancreatitis and diabetes. The toxicants in cigarette smoke can reach pancreatic tissue, and most of the toxicants require cytochrome P450 (P450)-mediated metabolic activation to exert their toxicity. Among all the human P450 enzymes, CYP2A13 is the most efficient enzyme in the metabolic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a major tobacco-specific toxicant and a suspected human carcinogen. It also metabolically activates 4-aminobiphenyl, another toxicant in cigarette smoke. Immunohistochemical analysis in this

study demonstrated that CYP2A13 was selectively expressed in the islets but not in the exocrine portion of adult human pancreas. Further study using dual immunofluorescence labeling technique showed that CYP2A13 protein was mainly expressed in the α -islet but not in β -islet cells. The selective expression of CYP2A13 in human pancreatic α -islet cells suggests that these islet cells could be damaged by the toxicants existing in cigarette smoke through CYP2A13-mediated in situ metabolic activation. Our result provides a mechanistic insight for human pancreatic diseases that have been associated with cigarette smoke exposure.

Introduction

Both epidemiological and experimental data have demonstrated that cigarette smoking is an etiological factor of pancreatic cancer and is associated with an increased risk for the development of chronic pancreatitis (Mulder et al., 2002; Maisonneuve et al., 2006; Wittel et al., 2006; Tranah et al., 2011). Recent studies indicated that cigarette smoking may also play an important role in the pathogenesis of diabetes. Passive smoking was found to be more frequent in children with type 1 diabetes or prediabetes (Hathout et al., 2006; Skrodeniene et al., 2008). In both healthy persons and patients with chronic pancreatitis, the serum glucose levels in smokers were substantially higher than their nonsmoker controls, whereas the insulin levels were lower (Milnerowicz et al., 2007). Mechanistic studies are needed to support these observations in humans and to establish an etiological and/or pathological role of tobacco smoke in pancreatic diseases.

There are many toxic and carcinogenic chemicals in cigarette smoke, including nicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N*-nitrosobis(2-oxopropyl)amine, and 4-aminobiphenyl (Hoffmann et al., 1993; Chowdhury et al., 1995; Standop et al.,

2001). These toxic chemicals can reach the pancreas, as demonstrated by their detection in the pancreatic juice from smokers and the nonsmokers who were exposed to second-hand tobacco smoke (Prokopczyk et al., 2002). Most of these toxicants and carcinogens require metabolic activation, usually catalyzed by cytochrome P450 (P450) enzymes, to generate the electrophilic metabolites that are responsible for their toxicity and carcinogenicity. The intermediate metabolites that are generated during the metabolic activation are very short-lived ($t_{1/2}$ often within a second or less). Therefore, it has been well accepted that P450-mediated metabolic activation in situ (i.e., in the target sites) is critical for cell- or tissue-specific toxicity or carcinogenicity induced by environmental chemicals (Ding and Kaminsky, 2003).

CYP2A13, CYP2A6, and CYP2A7 are three known members in the human CYP2A subfamily (Fernandez-Salquero et al., 1995). CYP2A7 has no catalytic activity, and CYP2A6 is a major human enzyme that catalyzes the metabolism of coumarin and nicotine (Ding et al., 1995). Our previous studies demonstrated that CYP2A13 is the most efficient human P450 enzyme for the metabolic activation of NNK, a tobacco-specific toxicant and suspected human carcinogen (Su et al., 2000). CYP2A13 can also metabolically activate 4-aminobiphenyl, another carcinogen in cigarette smoke (Nakajima et al., 2006). In addition, CYP2A13 is highly efficient in metabolizing nicotine (Bao et al., 2005) as well as the metabolic activation of aflatoxin B₁ (He et al., 2006), naphthalene, and styrene (Fukami et al., 2008).

Using a highly specific antibody for immunohistochemistry, we previously demonstrated a high level of CYP2A13 protein expression in the epithelial cells of human bronchus and trachea but not in human liver, heart, testis, and ovary (Zhu et al., 2006). The selective expres-

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ABBREVIATIONS: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; P450, cytochrome P450; FITC, fluorescein isothiocyanate; POR, P450 oxidoreductase.

sion of CYP2A13 protein in human respiratory tissue is consistent with the fact that most smoking-related human lung cancers are bronchogenic. It also supports the role of CYP2A13-mediated in situ activation of NNK and other toxicants in smoking-related human lung cancer. It is of interest to notice a recent report in which an inactive CYP2A13 variant was found to be associated with decreased pancreatic cancer susceptibility (Mohelnikova-Duchonova et al., 2010).

We speculated that CYP2A13, if it is expressed in pancreas, may play the same important role in smoking-related pancreatic diseases. This study aimed to determine the expression of CYP2A13 in human pancreas and to identify its cellular localization.

Materials and Methods

Antibodies and Reagents. Normal goat serum, biotinylated goat anti-rabbit IgG, antigen unmasking solution, Vectastain ABC reagent, and peroxidase substrate kit (DAB SK-4100) were purchased from Vector Laboratories Inc. (Burlingame, CA). Mouse anti-proinsulin C-peptide IgG, fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG, and rhodamine-conjugated goat anti-mouse IgG were obtained from Millipore (Billerica, MA). Mouse anti-glucagon IgG and Mayer's hematoxylin solution were purchased from Sigma-Aldrich (St. Louis, MO). Rabbit anti-CYP2A13 polyclonal antibody was generated in our laboratory (Zhu et al., 2006). This antibody was raised against a C-terminal CYP2A13-specific peptide sequence covering the amino acid residues 369 to 377. The selection of this antigenic peptide was based on the hydrophilicity and the side chain properties of the amino acid residues that differ most from either CYP2A6 or CYP2A7. The specificity of this antibody was validated by immunoblot and immunohistochemical analyses. It does not cross-react with any of the P450 proteins present in human liver microsomes, including CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP2E1, and CYP3A4, nor does the antibody cross-react with CYP2A6 and mouse CYP2A5, both of which share a significant protein sequence similar to CYP2A13 (Zhu et al., 2006).

Tissue Samples. Human pancreas samples were a kind gift from Department of Pathology, Tongji Medical College, Huazhong Science and Technology University, Wuhan, China. They were collected from individual patients during pancreatectomy, with the consent of patients and the approval of the local ethics committee. The sample data (including the age, gender, and pathological diagnosis of the patients) are available from the Department of Pathology, Tongji Medical College. Twelve samples (four pancreatic cancer tissues and eight normal pancreatic tissues adjacent to cancer) were collected and embedded in paraffin blocks.

Immunohistochemistry. Paraffin sections (5- μ m thick) were prepared from the paraffin blocks. The immunohistochemical analysis protocol was described in detail in our previous report (Zhu et al., 2006). The dilution of rabbit anti-CYP2A13 antiserum was 1:600. Staining specificity was appraised by substitution of the primary antibody with the preimmune serum from the same rabbits. The immunohistochemical staining was performed at least three times with several adjacent sections, and the results were confirmed independently by four of the authors (Y.G., L.-R.Z., G.L., and J.-Y.H.).

Dual Immunofluorescence Labeling. Tissue sections were first processed through the same steps as for immunohistochemistry analysis (de-paraffinization, hydration, wash, antigen retrieval, and blocking of nonspecific binding). Then, for dual labeling, sections were incubated with anti-CYP2A13 antiserum (diluted 1:600) mixed with either mouse anti-proinsulin C-peptide IgG (diluted 1:200) or mouse anti-glucagon IgG (diluted 1:2000) in a humidified chamber overnight at 4°C. After washing with phosphate-buffered saline, the sections were incubated with FITC-conjugated goat anti-rabbit IgG and rhodamine-conjugated goat anti-mouse IgG (diluted 1:200) for 1 h. Sections were washed in phosphate-buffered saline again and then mounted in glycerol.

Results and Discussion

We have used a highly specific anti-CYP2A13 antibody in immunohistochemical analysis to localize the expression of CYP2A13 protein in human pancreatic tissues. In all the eight normal samples, strong immunostaining was observed almost exclusively in the pancreatic islet cells, but it was very weak in acinar cells and was even

rare in ductal cells (Fig. 1A). CYP2A13-specific staining was undetectable in the negative controls (Fig. 1B), in which the anti-CYP2A13 antibody was replaced by the preimmune serum from the same rabbits. The specificity of CYP2A13 immunoreactivity was further evidenced by including additional controls (human bronchus and liver tissue sections) in the same immunohistochemistry experiment. CYP2A13-specific immunostaining was positive in human bronchial epithelial cells (Fig. 1C) but was absent in human liver (Fig. 1E). This is consistent with our previous reports that the expression level of mRNA and protein of CYP2A13 is high in human bronchial cells but has little or no expression in the human liver (Su et al., 2000; Zhu et al., 2006). There was no detectable CYP2A13 protein in four pancreatic cancer samples (data not shown), probably due to the absence of normal islet morphology in the cancer tissues.

The pancreatic islets consist of mainly α -cells (producing glucagon, 15–20% of total islet cells), β -cells (producing insulin, 65–80%), δ -cells (producing somatostatin, 3–10%), and PP cells (producing pancreatic polypeptide, 3–5%) (Cabrera et al., 2006). To identify which type(s) of the islet cells express CYP2A13 protein, we applied immunofluorescent double-staining assay in which anti-CYP2A13 antibody was combined with the antibody against proinsulin C-peptide, a distinct hormone secreted only by β -islet cells, or with the antibody against glucagon, a specific hormone synthesized in α -islet cells. Double staining results showed that, in all eight normal human pancreatic tissues, CYP2A13-expressing cells (labeled by FITC in green fluorescence) were in small quantities and mostly scattered in the peripheral islet region with some single cells in the central part of the islet (Fig. 2, A-1 and B-1). In contrast, insulin-expressing β -cells (labeled by rhodamine in red fluorescence), which accounted for approximately 70% of total islet cells, were mainly localized in the center of the islet (Fig. 2, A-2). Consistent with the known histological distribution of α -cells in pancreatic islets and the distribution of CYP2A13-expressing cells, glucagon-producing α -cells (also labeled by rhodamine in red fluorescence) were mainly localized at peripheral or perisinusoidal space (Fig. 2, B-2). Superimposition results of the labeling patterns clearly showed that CYP2A13 protein was mainly expressed in α -cells (Fig. 2, A-3 and B-3).

Expression of several other P450 enzymes, including CYP1A2, CYP2B6, CYP2C8/9/19, CYP2E1, CYP2J2, and CYP3A, and NADPH-cytochrome P450 oxidoreductase (POR), an essential supporter of P450-catalyzed reaction, in human pancreatic islets has been reported (Zeldin et al., 1997; Standop et al., 2002, 2003; Ulrich et al., 2002). Similar to CYP2A13, CYP2B6, CYP2C8/9/19, CYP2E1, CYP2J2, and CYP3A proteins are also mainly expressed in α -cells. The coexistence of these P450 and POR proteins in the islet cells indicates that the P450 enzymes in human pancreatic islets are functional in metabolizing various endogenous and exogenous substrates. This was supported by the presence of epoxyeicosatrienoic acids, the metabolites produced in CYP2J2-catalyzed reaction, in human pancreatic tissues (Zeldin et al., 1997).

Whereas CYP2J2 is known as an epoxygenase for catalyzing the metabolism of arachidonic acid (Zeldin et al., 1997), the endogenous substrates of most P450 enzymes are largely unknown. Therefore, it is difficult to understand the physiological role of P450 enzymes expressed in pancreatic islets. In contrast, the toxicological significance of P450 enzymes in the islets appears to be clear based on our current understanding of the critical role of P450-mediated metabolic activation in toxicity induced by environmental toxicants, such as CYP2A13-catalyzed activation of NNK and other toxicants in cigarette smoke (Wang et al., 2006). It is also worth mentioning that the islet cells are the first to expose to blood-borne toxicants as a large portion of arterial blood passes through the islets first in pancreatic

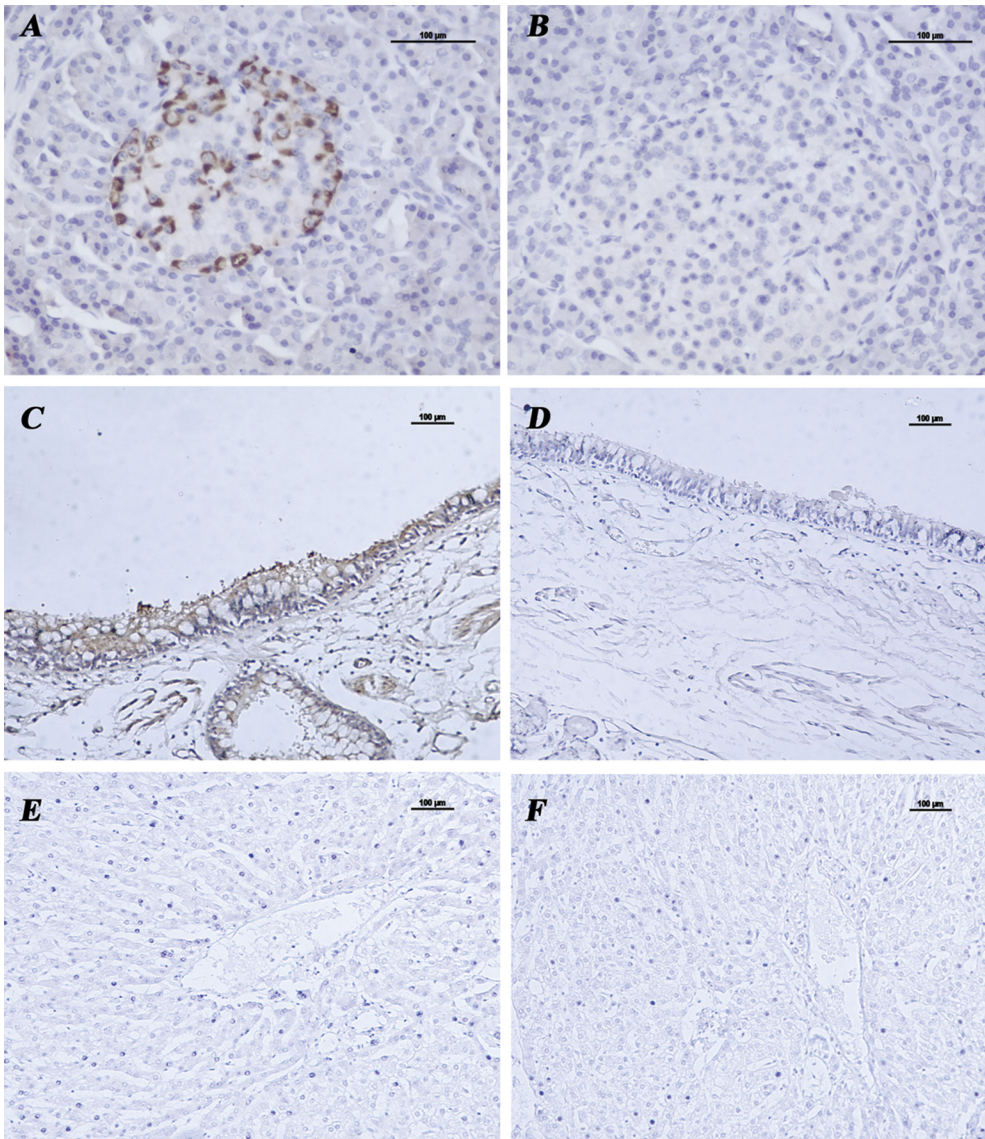


FIG. 1. Immunohistochemical localization of CYP2A13 protein in adult human pancreas, bronchus, and liver. A and B, pancreas; C and D, bronchus; E and F, liver. Anti-CYP2A13 antibody was used in A, C, and E. Preimmune rabbit serum, as a negative control, was used in B, D, and F. The magnification for A and B is 200 \times ; the magnification for C, D, E, and F is 100 \times .

blood supply (Standop et al., 2002). The high P450 expression and exposure priority would make pancreatic islet a vulnerable target of toxicants that reach pancreas and undergo the metabolic activation in situ.

Metabolic activation of tobacco-specific toxicant NNK by CYP2A13 in α -cells is anticipated to cause cell damage. This notion is supported by a recent study that demonstrated the adverse effect of cigarette smoking on α -cells. It has been found that the number of pancreatic α -cells in patients with chronic pancreatitis was significantly decreased in smokers compared with patients who did not smoke and healthy subjects (Milnerowicz et al., 2007). The association of smoking and α -cell damage was further confirmed by the finding that fibrosis and progressive atrophy of α -cells were observed in patients who smoked and had chronic pancreatitis, but the pancreatic islets were normal in patients who did not smoke (Milnerowicz et al., 2007).

The damage induced by toxicants in tobacco smoke on α -cells may also lead to damage in β -cells. In human pancreatic islets, α -cells and β -cells are in close contact (Cabrerria et al., 2006). An early study found that morphologically typical tight and gap junctions occur between α - and β -cells (Orci et al., 1975). It has also been demonstrated that carboxyfluorescein (an impermeant fluorescent probe),

after microinjection into the β -cells, can be directly transferred to neighboring α -cells and δ -cells (Meda et al., 1982). Therefore, it is reasonable to expect that the toxic metabolites generated inside α -cells could be transferred directly by gap junctions from α -cells to neighboring β -cells, causing the β -cell damage and death. In addition to this "bystander effect," it has been well known that different types of pancreatic islet cells can interact with each other through a paracrine feedback system. Most β -cells express glucagon receptors (Kieffer et al., 1996). Glucagon modulates α - and β -cell proliferation in the establishment of islet phenotype during pancreas development (Vuguin et al., 2006). Whereas insulin produced in β -cells can inhibit α -cells, glucagon produced in α -cells activates β -cells (Wang et al., 2001). Therefore, reduction in the number of α -islet cells and impaired excretion of glucagon may induce loss or dysfunction of β -cells. Indeed, loss of β -cells has been observed in patients who were smokers and had chronic pancreatitis (Milnerowicz et al., 2007), and deficiency of glucagon was found to be associated with pancreaticogenic diabetes mellitus (Goldstein et al., 1989).

In conclusion, we demonstrated that CYP2A13 protein is almost exclusively expressed in the α -islet cells in human pancreas. The expression of CYP2A13 protein in pancreatic islet cells was also observed in human fetuses (Y. Guo, W. Meng, Y. Wu, J.-Y. Hong,

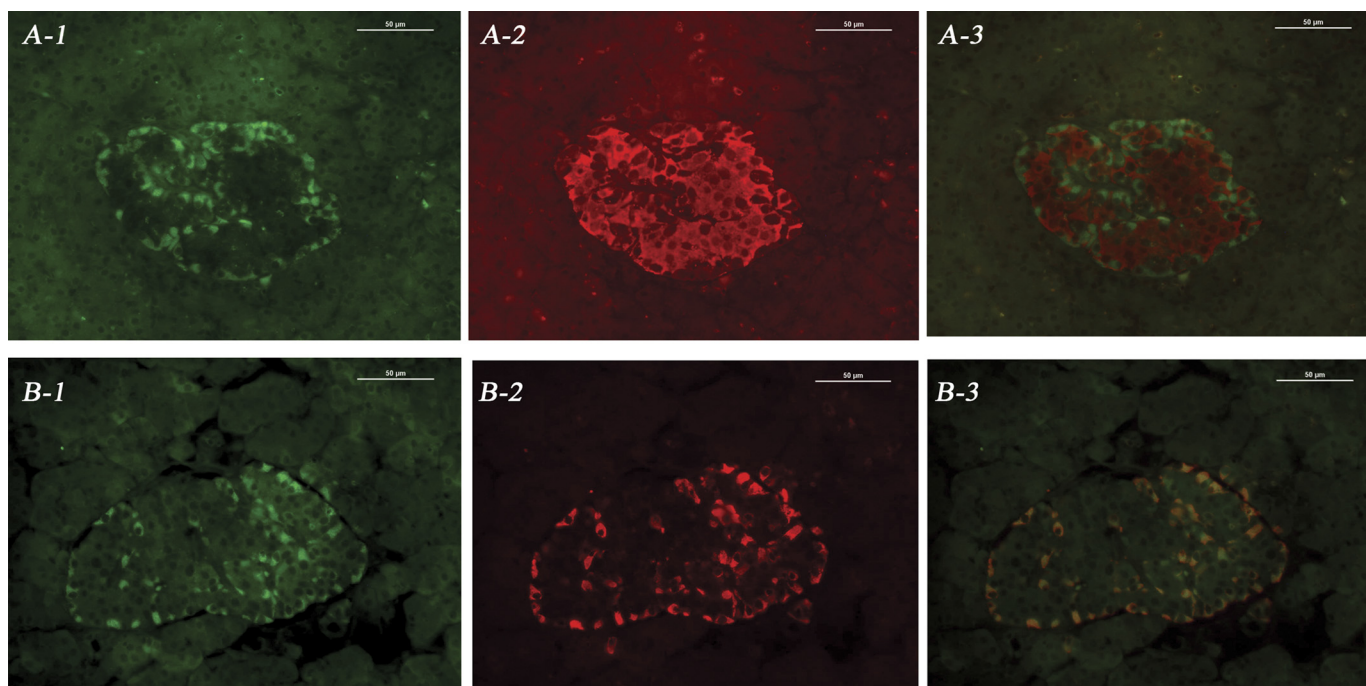


FIG. 2. Immunofluorescent double staining images of CYP2A13, proinsulin C-peptide, and glucagon in adult human pancreatic islets (magnification, 400 \times). A-1 and B-1, rabbit anti-CYP2A13 antibody and FITC-conjugated goat anti-rabbit IgG were used as the primary and secondary antibody, respectively; A-2, mouse anti-proinsulin C-peptide IgG and rhodamine-conjugated goat anti-mouse IgG were used as the primary and secondary antibody, respectively; A-3, superimposition of A-1 and A-2; B-2, mouse anti-glucagon IgG and rhodamine-conjugated goat anti-mouse IgG were used as the primary and secondary antibody, respectively; B-3, superimposition of B-1 and B-2.

and H. Wang, unpublished data). The presence of a highly efficient enzyme for the metabolic activation of some toxicants in cigarette smoke in the pancreas suggests that CYP2A13-mediated activation plays an important role in pancreatic diseases induced by or associated with smoking.

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Authorship Contributions

Participated in research design: Guo and Hong.
Conducted experiments: Guo.
Wrote or contributed to the writing of the manuscript: Guo, Wang, and
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Other: Guo, Zhu, Lu, and Hong.

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