Expression of cytochrome P450 arachidonic acid epoxygenase 2J2 in human tumor tissues and cell lines

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Background and Objective: Cytochrome P450 arachidonic acid epoxygenase 2J2 (CYP2J2) is a new metabolic pathway of arachidonic acid. However, its biological effects, especially pathophysiologic significance in human beings, remain to be further recognized. This study was to determine the expression of CYP2J2 in human tumor tissues and cell lines. Methods: The expression of CYP2J2 mRNA and protein in 130 specimens of human carcinoma and related adjacent normal tissues, four specimens of inflammatory pseudotumor tissues, eight human tumor cell lines and two normal cell lines (as control) was detected by reverse transcription-polymerase chain reaction (RT-PCR), western blot and immunohistochemistry. Results: CYP2J2 was highly expressed in 101 (78%) carcinoma tissues, but was not detected in adjacent normal tissues and inflammatory pseudotumor tissues. Its mRNA level was obviously correlated to its protein level (r = 0.613, p < 0.01). Immunohistochemistry analysis showed the same results as RT-PCR and western blot. Furthermore, CYP2J2 was only expressed in cancer cells but not in interstitial and inflammatory cells. CYP2J2 was highly expressed in all carcinoma cell lines, but not in two normal cell lines. Conclusion: CYP2J2 is highly and selectively expressed in human tumor tissues and cell lines and may be a novel biomarker of human tumors.

As a prevalent disorder, tumors afflict humans with poor prognosis and high mortality. No effective biological marker is currently available for early diagnosis of tumors although some factors, such as diet, activation of c-myc oncogene and inactivation of p53 suppressor gene, are associated with increased incidence of tumors to some extent.1,2 Furthermore, the etiology and pathology of tumors remain unknown.

Recent studies have showed that cytochrome P450 isozymes CYP1A, CYP3A and CYP2C are closely associated with pathogenesis and progression of tumors.3,4 As a member of P450 isozyme family, cytochrome P450 arachidonic acid epoxygenase 2J2 (CYP2J2) extensively distributes in the heart, liver, lungs, kidneys, intestines, pancreas and vessels, mostly enriched in the heart and vascular epithelial cells. Its expression in tumors is also validated by some studies.3,5 These results indicate that CYP2J2 plays a critical role in pathogenesis and progression of cancer. In this study, we investigated the expression and significance of CYP2J2 in tumor tissue.

Materials and Methods

Specimens. A total of 130 paired fresh specimens of tumor tissues and adjacent normal tissues (including 31 cases of esophageal squamous carcinoma, four cases of esophageal adenocarcinoma, 37 cases of lung squamous carcinoma, 26 cases of lung adenocarcinoma, eight cases of small cell lung carcinoma, five cases of breast carcinoma, five cases of gastric carcinoma, ten cases of hepatocellular carcinoma and four cases of colon carcinoma) were obtained from hospitalized patients in Tongji Hospital from 2001 to 2002 with informed consent. All cases were pathologically diagnosed as malignant tumor. Of the 130 patients, 78 were men and 52 were women, with the median age of 52 years. Another four paraffin specimens of inflammatory pseudotumor were also enrolled. After sampling, some specimens were frozen in liquid nitrogen and subsequently stored at -80°C freezer, while the remaining were fixed in 10% formaldehyde and embedded in paraflin for consequent hematoxylin-eosin (HE) staining and immunohistochemical staining.

Materials. Trizol kit was purchased from Life Technologies (Gibco Co.). Rabbit anti-human CYP2J2 polyclonal antibody was gifted by Professor Capdevila, Vanderbilt University. Horseradish peroxidase-labeled goat anti-rabbit IgG was purchased from KPL Co. and ECL chemiluminescent reagent was from Pierce Co. SDS-PAGE standard indicator was purchased from Bio-Rad Co. SP kit and DAB substrate kit were purchased from Zhongshan Biotechnology, Beijing.

Cells and cell culture. Human colon carcinoma cell line LS-174, bladder cancer cell line ScaBER, cervical cancer cell line SiHa, astrocytoma cell line U251, lung cancer cell line A549, tongue squamous cancer cell line Tca-8113, small cell lung cancer

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Total RNA was extracted and purified by using Trizol kit, and further quantified. Primers were designed according to CYP2J2 and GAPDH (internal control) cDNA sequences from GenBank. The sequences for CYP2J2 upstream primer was 5’-TATCATGCTCGGCGATGG-3’ and for downstream primer was 5’-AAGCGTTCTCGGAAGGTG-3’; the sequences for GAPDH upstream primer was 5’-CCTTGCTCTCAGACATGC-3’ and for downstream primer was 5’-CCAGACATACTCAGC-3’. Total RNA (1 μg) was successively supplemented with 0.5 μL RNase inhibitor, 2 μL dNTP (10mmol/L), 1 μL AMV reverse transcriptase, 1 μL random primer, 2 μL 10 × RT-PCR buffer and 2 μL MgCl₂ (25 mmol/L), and added with water till a total volume of 20 μL. Reverse transcription was performed at 30 °C for 10 min and at 45°C for 30 min, and finally inactivated at 95 °C for 5 min. RT products (5 μL) were successively supplemented with 10 × PCR buffer, sense and antisense primers, and Taq DNA polymerase, and added with water till a total volume of 25 μL. Polymerase chain reaction (PCR) were performed as follows: pre-denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s and extending at 72°C for 1 min, with final extending at 72°C for 5 min. The size of PCR products were 600 bp for CYP2J2 and 310 bp for GAPDH. Target band density was analyzed by using gel imaging system.

Western blot test of CYP2J2 protein in tumor tissues and cells. Total proteins were extracted and purified by using Trizol kit. Protein samples (20 μg) were added with 3× sampling buffer, and boiled for 3 min, then loaded for vertical electrophoresis on prepared SDS-PAGE gel for 1.5–2.0 h. Gels of target protein bands were retrieved and proteins were transferred onto PVDF membrane at 25 V by using electric trans-blotter. The membrane was blocked with TBS-T containing 5% fat-free milk powder at room temperature for 2 h, incubated with primary antibody (1:500) at 4°C overnight. On the next day, the membrane was rinsed with TBS-T at room temperature for four times (15 min for each time), added with horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:8000) and gently shaken at room temperature for 2 h, then rinsed with TBS-T again at room temperature for four times (15 min for each time), finally added with ECL reagent and incubated at room temperature for 2–4 min, followed by exposure. Target bands were scanned with gel imaging system for density analysis.

Immunohistochemical staining. SP immunohistochemistry was performed according to kit instructions. The sections were dewaxed and rehydrated, followed by blocking of endogenous peroxidase with 3% H₂O₂ for 10 min, microwaved for 2 min to retrieve antigens, and incubated with CYP2J2 primary antibody at 4°C overnight, then rinsed with PBS (pH 7.4) for three times (5 min for each time) and incubated with biotinized secondary antibody at 37°C for 2 h, rinsed with PBS for three times (5 min for each time) and incubated with horseradish peroxidase-labeled streptavidin working solution for 40 min, rinsed with PBS for three times (5 min for each time) and colorized with DAB for 1–3 min, counterstained with hematoxylin, dehydrated, differentiated, mounted with neutral gum and observed under microscope. Primary and secondary antibodies were omitted for negative control. Cells with brownish staining in cytoplasm were considered as positive; while those without brownish staining or consistent with background staining were negative. Five visual fields of high magnification were randomly designated on each section to count 100 cells. The staining was further classified into grade 0–3 in contrast to background staining of normal tissue cells (grade 0 as negative, grade 1 as weak positive, grade 2 as positive and grade 3 as strong positive).³

Statistical analysis. The data were compared with Chi-square test and p < 0.05 was considered as significant. Logistic regression analysis was used for analysis of correlation.

Results

Expression of CYP2J2 mRNA in tumor tissues and matched adjacent normal tissues detected by RT-PCR. GAPDH was used as internal control. CYP2J2 mRNA was detected in 101 out of 130 tumor specimens with a positive rate of 77%, but was not detected in adjacent normal tissues (p < 0.01) (Fig. 1). The mRNA level of CYP2J2 was 0.4–1.8-fold higher in tumor tissues (1.29 ± 0.03) than in adjacent normal tissues (0.02 ± 0.01).

Expression of CYP2J2 protein in tumor tissues and matched adjacent normal tissues detected by western blot. β-actin was used as internal control. The positive rate of CYP2J2 protein was 78% in tumor tissues, which was significantly higher than...
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that in adjacent normal tissues (p < 0.01). The protein level of CYP2J2 was 0.5–1.8-fold higher in tumor tissues (1.34 ± 0.02) than in adjacent normal tissues (0.02 ± 0.02) (Fig. 2). Moreover, the expression of CYP2J2 mRNA was positively correlated to the expression of CYP2J2 protein (r = 0.613, p < 0.01).

Expression of CYP2J2 protein in tumor tissues and matched detected by tissues detected by immunohistochemistry. Only weak positive staining of CYP2J2 was observed in three specimens of adjacent normal tissues; positive staining of CYP2J2 was observed in 101 (78%) specimens of tumor tissues, including 22 specimens of esophageal squamous carcinoma, two specimens of esophageal adenocarcinoma, 29 specimens of lung squamous carcinoma, 17 specimens of lung adenocarcinoma, five specimens of small cell lung carcinoma, five specimens of breast carcinoma, ten specimens of hepatocellular carcinoma, five specimens of gastric carcinoma and four specimens of colon carcinoma. The variation in positive rate of CYP2J2 among different cancers was insignificant due to inadequate samples. The strong positive rate of CYP2J2 in tumor tissues was 55% (72/130), which was significantly higher than that in adjacent normal tissues (p < 0.01). CYP2J2 was not detected in organs of patients without tumor and in the four specimens of inflammatory pseudotumor. Besides less expression in tumor vascular endothelial cells, CYP2J2 was mainly expressed in tumor cells (Fig. 3).

Expression of CYP2J2 mRNA in tumor cells detected by RT-PCR. Based on the aforementioned results, eight common human-derived tumor cell lines (LS-174, ScaBER, SiHa, U251, A549, Tca-8113, Ncl-H446 and HepG2) were selected for RT-PCR test of CYP2J2; HEK293 and HT-1080 cells were used as control. The results were similar to what had been observed in tumor tissues. CYP2J2 was highly expressed in all the eight tumor cell lines, especially in ScaBER, SiHa, A549 and Tca-8113, while CYP2J2 was not expressed in HT-1080 and HEK293 cells (Fig. 4A).

Expression of CYP2J2 protein in tumor cells detected by western blot. Consistent with RT-PCR results, CYP2J2 protein was expressed in tumor cell lines to various extent, but not expressed in HT-1080 and HEK293 cells (Fig. 4B). The cell lines with higher expression of CYP2J2 mRNA also presented higher expression of CYP2J2 protein, suggesting that CYP2J2 mRNA stably existed in tumor cells and translated into protein by a process that is not yet fully understood.

Discussion

Investigation on CYP epoxygenase has been popular recently due to the extensive physiologic effects of its metabolites, especially their regulatory effects on inflammation and blood pressure. Moreover, CYP epoxygenase is closely associated with cellular signal transduction, mitosis, apoptosis and oncogenesis. CYP epoxygenase consists of 2C and 2J. CYP2C was reported to be expressed in humans, while six genes of 2J family have been cloned, while CYP2J2 has been detected in humans.

Epidemiological studies confirmed that COX-2 and 5-lipoxygenase metabolizing arachidonic acids are both associated with oncogenesis, whose potential acts through multiple mechanisms. It is most likely that their high expression would result in proliferation of tumor cells but inhibit apoptosis of tumor cells instead. As a new metabolic pathway of arachidonic acids, the in vivo biological and pathophysiological roles of CYP450 need to be characterized. Expression of CYP epoxygenase in tumor tissues was hereby studied for designing novel therapeutic and diagnostic strategies prospectively.

In the study, CYP2J2 was highly and specifically expressed in tumor tissues with a positive rate of 78%, while it couldn’t be detected by western blot (B) analysis of CYP2J2 in 10 cell lines. Lane 1, LS-174 cells; lane 2, ScaBER cells; lane 3, SiHa cells; lane 4, U251 cells; lane 5, A549 cells; lane 6, Tca-8113 cells; lane 7, Ncl-H446 cells; lane 8, HepG2 cells; lane 9, HT1080 cells; lane 10, HEK293 cells; lane M, marker.
detected in adjacent normal tissues; its mRNA expression was positively correlated to its protein expression. Further immunohistochemical analysis revealed that CYP2J2 was mainly expressed in tumor cells but negative in surrounding inflammatory cells and stromal tissues, and it was detected in vascular epithelia cells of tumor tissues and adjacent normal tissues; while in four specimens of actively proliferating inflammatory pseudotumor, CYP2J2 was undetectable. These results suggest that CYP2J2 is selectively and highly expressed in human tumor cells. Recently, Gaedigk et al. reported that CYP2J2 mRNA was detected in the liver and heart at embryonic 11th week and remained constant till birth, but significant variation in CYP2J2 protein expression was observed among individuals due to polymorphisms of CYP2J2 gene. In our study, CYP2J2 was only detected in three specimens of normal tissues, probably due to less expression of itself in tissues, which was undetectable with the methods used in our study.

To validate the universality of CYP2J2 expression in tumor cells, eight common tumor cell lines (LS-174, ScabER, SiHa, U251, A549, Tca-8113, Ncl-H446 and HepG2) were selected for RT-PCR and western blot to detect expression of CYP2J2. CYP2J2 was highly expressed in all the eight tumor cell lines, but not expressed in control HT-1080 and HEK293 cells, indicating its cellular selectivity and its potential role as a malignancy marker.

In vivo function of CYP2J2 in pathogenesis and progression of malignancies remains unclear. Its potential oncogenesis might act through multiple mechanisms with the most likelihood that high expression of CYP2J2 results in elevation of EETs in tumor tissues. As a differentiating and growth factor, EETs enhances not only proliferation of endothelial cells but also formation of endothelial capillary-like structures, whereby tumor growth highly depends on nutritional supply from vessels. Some other evidences testified that CYP450 could accelerate metabolism of anti-tumor substance, thus facilitating oncogenesis and progression in a favorable biological microenvironment. Gaedigk et al. reported that CYP2J2 mRNA was detected in human fetal tissues; while in four specimens of human tumor tissues and adjacent normal tissues; its mRNA expression was positive correlated to its protein expression. Further immunochemical analysis revealed that CYP2J2 was selectively and its potential role as a malignancy marker.

In summary, CYP2J2 mRNA and protein are highly expressed in tumor cells, indicating that CYP2J2 may be a molecular marker of some tumors, which can be used in pathologic diagnosis, dynamic observation, and prognosis estimation for malignancies. Irregular follow-up is recommended for the patients with high expression of CYP2J2.

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References