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Impact of hepatic malignancy on CYP3A4 gene expression

Bridget N. Fahy, MD,[a,*] *Tao Guo, MS,*[b] *and Romi Ghose, PhD*[b]

[a] *Department of Surgery, Weill Cornell Medical College, The Methodist Hospital, Houston, Texas*
[b] *Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, Texas*

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ABSTRACT

Background: The aim of this study was to examine differences in a major enzyme system for hepatic metabolism of drugs, CYP3A4, by measuring RNA levels in the liver tissue of subjects with and without hepatic malignancy and with primary *versus* metastatic liver tumors.

Materials and methods: We identified liver specimens from a hospital-wide tissue repository of patients having liver resection for a clinical indication. Total RNA isolation, complementary DNA synthesis, and real-time quantitative polymerase chain reaction were performed according to the standards. Demographic, clinical, and laboratory data were obtained from medical records. Standard statistical analyses were performed with significance set to $\alpha = 0.05$.

Results: Liver tissue from 27 subjects was available for analysis: 13 were without malignancy and 14 had either primary liver malignancies ($n = 7$) or metastatic disease ($n = 7$). Median age was 57 y, and half of the subjects were men. More than 80% of subjects were overweight or obese without differentiation between benign or malignant tumors. Fewer than 20% of subjects had diabetes or hypercholesterolemia. No preresection laboratory differences were noted between the groups (benign *versus* malignant or primary *versus* metastatic disease). Subjects with malignant liver tumors had significantly lower relative-fold CYP3A4 RNA content than those with benign liver tumors ($P = 0.009$), but no difference in the CYP3A4 RNA content between primary and metastatic disease was seen.

Conclusions: This study demonstrates differences in the expression of CYP3A4 in benign and malignant human liver tumors and contributes to understanding the possible impact of malignancy on hepatic metabolism.

1. Introduction

Drug metabolism is primarily accomplished through specialized enzymatic systems in the liver. The rate of metabolism is an important determinant of the duration and intensity of the pharmacologic action of drugs. Perturbations in the normal rate of metabolism can lead to serious consequences in both directions; metabolism that is too rapid can lead to subtherapeutic dosing of drugs and clinical inefficacy, whereas metabolism that is too slow can lead to toxic levels of a drug

* *Corresponding author*. Department of Surgery, Weill Cornell Medical College, The Methodist Hospital, 6550 Fannin Street, SM1661, Houston, TX 77030. Tel.: +1 713 441 5177; fax: +1 713 790 6472.
E-mail address: bnfahy@tmhs.org (B.N. Fahy).
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and/or alterations in the metabolism of other drugs administered at the same time. This can lead to drug–drug interactions and adverse drug reactions, including drug-induced liver injury. Drug-induced liver injury is the leading cause of acute liver failure in the United States, accounting for approximately 40% of cases of acute liver failure [1]. It is also a major reason for discontinuing certain drugs.

Recently, there has been an increased attention to the interaction between malignancy and liver metabolism as clinicians and patients seek to maximize chemotherapeutic efficacy while minimizing toxicity. The challenge in balancing chemotherapy efficacy and toxicity is well illustrated by the chemotherapy-associated liver injury reported among patients undergoing liver resection for colorectal metastases who received systemic chemotherapy before resection. In a recent study by Vauthey *et al.* [2], patients who received the chemotherapeutic agent irinotecan had a five-fold risk of having steatohepatitis in their liver resection specimens compared with those who did not receive chemotherapy before liver resection. Furthermore, patients with steatohepatitis had a 10-fold increased 90-d mortality compared with those who did not have steatohepatitis. In this instance, not only was chemotherapy found to be associated with liver injury but also this injury resulted in significantly higher postoperative mortality after liver resection.

Drug metabolism also has an important impact on chemotherapeutic antitumoral effects. Certain antitumoral agents (e.g., tamoxifen) require extensive metabolism by cytochrome P450 (CYP) enzymes to elicit their antitumor effects [3]. Furthermore, it has been shown that certain metabolites of these drugs are generated by different members of the CYP enzyme family, which leads to potential differences in drug levels after administration of such agents depending on genetic variations in the presence and activity of such hepatic metabolizing enzymes [4,5]. Genetic variations in CYP also have important implications for cancer-related outcomes as shown in a recent study by Goetz *et al.* [6] who demonstrated that women with a certain null allele of CYP2D6 were poor metabolizers of tamoxifen and had shorter time to relapse of their breast cancer and worse disease-free survival compared with those with other CYP2D6 genotypes.

Although a number of drug-metabolizing enzymes are involved in hepatic metabolism, we focused on CYP, and specifically CYP3A4 in the present study, because it is one of the most important enzymes involved in the metabolism of therapeutic drugs (including many classes of anticancer drugs) [7,8]. It is involved in the oxidation of the largest range of substrates of all the CYPs and is present in the largest quantity of all the CYPs in the liver. Furthermore, the extensive polymorphisms in this gene may prove important in the future prediction of drug toxicity, resistance, or improved therapeutic response to drugs metabolized by these genes.

The aim of the present study was to examine differences in CYP3A4 RNA levels in liver tissue specimens obtained from subjects with and without underlying malignancy to identify possible alterations in drug hepatic metabolism that might be expected in those with and without malignancy. We also sought to identify differences in CYP3A4 gene expression between subjects with primary *versus* metastatic liver tumors.

2. Materials and methods

Twenty-seven liver specimens were identified from our Institutional Review Board–Approved Tissue Repository at The Methodist Hospital Research Institute. Samples were chosen based on the presence or absence of malignancy within the liver specimen. Separate institutional review board approval was obtained to perform a retrospective chart review to collect demographic, clinical, and laboratory data to correlate with the final pathologic diagnosis of benign, primary hepatic malignancy, and metastatic liver tumors.

All liver specimens were obtained from patients undergoing liver resection for a clinical indication (e.g., diagnosis and oncologic resection). Because the specimens were drawn from a tissue repository, it was not possible to determine the precise location of the liver sample in relation to the liver tumor. However, all the specimens used in the present study were taken from liver tissue adjacent to the resected tumor and were not tumoral samples. After the completion of standard clinical pathologic evaluation, specimens for tissue banking were placed in a liquid nitrogen bath and snap frozen. The specimens were stored in freezers maintained at −80°C.

2.1. *Total RNA isolation and complementary DNA synthesis*

Total RNA was isolated from human liver tissues using TRI Reagent (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. Briefly, the tissues were homogenized in the TRI Reagent, followed by incubation with chloroform. Total RNA was precipitated with isopropyl alcohol and suspended in diethylpyrocarbonate-treated water, followed by digestion with recombinant bovine DNase I (Applied Biosystems, Inc, Foster City, CA). Complementary DNA was synthesized using "High Capacity Reverse Transcription Kit" from Applied Biosystems according to the manufacturer's instructions.

2.2. *Real-time quantitative polymerase chain reaction analysis*

Real-time quantitative polymerase chain reaction was performed using an ABI PRISM 7300 Sequence Detection System instrument and software (Applied Biosystems, Inc) as described previously [9,10]. In brief, each amplification reaction (25 μL) contained 50–100 ng of complementary DNA, 300 nM of forward primer, 300 nM of reverse primer, 200 nM of fluorogenic probe, and 12.5 μL of TaqMan Universal PCR master mix (Applied Biosystems, Inc). The forward primer sequence of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was 5′-CATGGGTGTGAACCATGAGAA-3′. The reverse primer sequence of GAPDH was 5′-GGTCATGAGTCCTTCCACGAT-3′. The probe sequence of GAPDH was 5′-AACAGCCTCAAGATCATCAGCAATGCCT-3′. The sequences of the primers and probe were obtained from Sigma. TaqMan Gene Expression Assays of CYP3A4 (Cat #Hs00430021_m1) was purchased from Applied Biosystems. Quantitative expression values were extrapolated from standard curves and were normalized