Tumor-specific Expression of Cytochrome P450 CYP1B1

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ABSTRACT

Cytochrome P450 CYP1B1 is a recently cloned dioxin-inducible form of the cytochrome P450 family of xenobiotic metabolizing enzymes. An antibody raised against a peptide specific for CYP1B1 was found to recognize CYP1B1 expressed in human lymphoblastoid cells but not to recognize other forms of cytochrome P450, particularly CYP1A1 and CYP1A2. Using this antibody, the cellular distribution and localization of CYP1B1 were investigated by immunohistochemistry in a range of malignant tumors and corresponding normal tissues. CYP1B1 was found to be expressed at a high frequency in a wide range of human cancers of different histogenetic types, including cancers of the breast, colon, lung, esophagus, skin, lymph node, brain, and testis. There was no detectable immunostaining for CYP1B1 in normal tissues. These results provide the basis for the development of novel methods of cancer diagnosis based on the identification of CYP1B1 in tumor cells and the development of anticancer drugs that are selectively activated in tumors by CYP1B1.

INTRODUCTION

Cytochrome P450s are a multigene family of constitutive and inducible enzymes that have a central role in the oxidative metabolic activation and detoxification of both a wide range of xenobiotics (1–5) and several groups of endogenous compounds that are active in cell regulation and cell signaling, including arachidonic acid (6), steroid hormones (7), and fatty acids (8). The major families of cytochrome P450 involved in xenobiotic metabolism each consist of several individual forms with different regulatory mechanisms and substrate specificities (1). The majority of cytochrome P450s are primarily expressed in the liver (1, 4), although individual cytochrome P450 forms are also expressed in specific extrhepatic tissues, including small intestine, kidney, and lung (9–11).

The human CYP1 (individual cytochrome P450 forms are identified by the prefix CYP in accordance with the current cytochrome P450 nomenclature as described in Ref. 2) gene family, which is one of the major cytochrome P450 families involved in the metabolism of xenobiotics, is now known to consist of three individual forms classified into two subfamilies. The CYP1A subfamily contains two highly homologous and well-characterized but distinct members, CYP1A1 (12) and CYP1A2 (13). CYP1A1 is an inducible cytochrome P450 expressed primarily in extrhepatic tissues (14), whereas CYP1A2 is a major form of cytochrome P450 that is expressed constitutively in liver (15). Recently, a second human CYP1 subfamily has been identified, which to date contains one member, CYP1B1 (16). This cytochrome P450 is dioxin inducible, and sequence analysis of CYP1B1 shows 40% homology with both CYP1A1 and CYP1A2. Orthologous CYP1B1 forms have also been cloned recently from mouse (17) and rat (18, 19).

Several forms of cytochrome P450 are considered to have an important role in tumor development because they can metabolize many potential carcinogens and mutagens (20–23). Moreover, cytochrome P450 activity may influence the response of established tumors to anticancer drugs because several cancer chemotherapeutic agents can be either activated or detoxified by this enzyme system (22, 24). The presence of individual forms of cytochrome P450 has previously been investigated in different types of cancer, including breast (25), lung (26, 27), colon (28–31), and head and neck (32) cancer to determine whether intratumor metabolism of anticancer agents by cytochrome P450 could occur and thus influence the response of tumors to these agents. These studies have generally shown that the level of the cytochrome P450 forms investigated is significantly reduced or absent in tumors when compared with the adjacent normal tissue in which the tumors have developed. However, other studies, including studies in our laboratories, of several different types of cancer, including breast cancer (33), esophageal cancer (34, 35), and soft tissue sarcomas (36), have shown that there is expression of a CYP1 form of cytochrome P450 in these tumors. We have recently found that CYP1B1 mRNA is the most frequently expressed form of the CYP1 family mRNA in breast cancer (37), suggesting strongly that CYP1B1 is the main form of the CYP1 family present in breast cancer (37). In this study, we show that CYP1B1 shows enhanced expression in a wide range of malignant tumors and is not detectable in normal tissues.

MATERIALS AND METHODS

Tissue Samples. Samples of cancer and normal tissue from a wide range of tissues, including bladder, breast, colon, esophagus, kidney, lung, ovary, skin, stomach, uterus, connective tissue, lymph node, brain, and testis, were obtained from tissue specimens that had been submitted for diagnosis to the Department of Pathology, University of Aberdeen. These samples were from patients undergoing surgery for different types of malignant disease. For all tissues except lymph node, paired samples of normal and tumor tissues were available from each specimen of tissue. Normal lymph nodes were obtained from tissue samples removed for nontumor reasons. Normal liver and small intestine were also obtained with permission from organ transplant donors. For immunohistochemistry, the tissue samples had all been fixed in 10% neutral buffered formalin for 24 h at room temperature, and then blocks of both macroscopically normal and macroscopically tumor tissue were selected for histopathological analysis. These tissue blocks were then embedded in wax, and one section from each block was stained with H&E. These sections were examined by light microscopy, and histopathological diagnosis was made using established histological criteria. Samples of normal liver and breast cancer were also obtained fresh (not fixed in formalin), and these samples were used to prepare microsomes as described previously (38).

Anti-CYP1B1 Antibody. A new, specific, anti-CYP1B1 antibody was produced using a synthetic peptide corresponding to amino acids 332–345 of the deduced CYP1B1 amino acid sequence (39). This 14-amino acid peptide sequence was selected by comparing the CYP1B1 sequence with those of CYP1A1 and CYP1A2 and is specific for CYP1B1. A COOH-terminal cys-
RESULTS

CYP1B1

CYP1B1 antibody. The anti-CYP1B1 antibody that was generated using the synthetic peptide corresponding to the CYP1B1 specific sequence (Fig. 1) recognized expressed CYP1B1 (Fig. 2) and a single band of identical molecular size (M, 52,000) in breast cancer microsomes. The antibody did not recognize expressed CYP1A1 or any protein in human liver microsomes. The liver microsomes have been shown previously to contain CYP1A2 (41).

Immunohistochemistry. Immunohistochemistry for CYP1B1 showed that CYP1B1 immunoreactivity was detected in all of the different types of cancer studied and that there was strong CYP1B1 immunoreactivity in every type of tumor (Table 1; Figs. 3 and 4). In each type of tumor, CYP1B1 was expressed at high frequency, and only in 5 of 127 tumors was CYP1B1 not detected. In every type of cancer, CYP1B1 immunoreactivity was localized specifically to tumor cells (Figs. 3 and 4); nontumor cells, including inflammatory cells and endothelial cells present in the sections of tumor, showed no apparent immunoreactivity for CYP1B1. There was no significant intratumor heterogeneity of CYP1B1 immunoreactivity and no detectable immunoreactivity for CYP1B1 in any of the normal tissues studied, including liver, kidney, and small intestine (Table 1; Fig. 3).

DISCUSSION

The absence or low levels of individual forms of cytochrome P450 in many studies of human cancer (25-32), combined with extrapolation of a 1:2:3:4 ratio of CYP1B1:1:2:3:4

- 205kDa
- 116kDa
- 97kDa
- 58kDa
- 40kDa
- 30kDa

Fig. 2. Immunoblot of microsomes from human lymphoblastoid cells expressing CYP1B1 (Lane 1), human breast cancer microsomes (Lane 2), microsomes from human lymphoblastoid cells expressing CYP1A1 (Lane 3), and normal human liver microsomes (Lane 4). The anti-CYP1B1 antibody recognizes recombinant CYP1B1 and CYP1B1 present in breast cancer microsomes and does not recognize either expressed CYP1A1 or any protein in liver microsomes. Ten μg of protein were loaded per lane; in Lane 1, this corresponds to 0.74 pmol of expressed CYP1B1. Right, position of molecular weight standards.

3 The abbreviation used is: TBS, Tris-buffered saline.
TUMOR-SPECIFIC EXPRESSION OF CYPIB1

Table 1 Expression of CYPIB1 in different types of malignant tumors and normal tissue

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal (No. positive/no. tested)</th>
<th>Tumor [no. positive/no. tested (histopathological diagnosis)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>0/8</td>
<td>8/8 (transitional cell carcinoma)</td>
</tr>
<tr>
<td>Brain</td>
<td>0/12</td>
<td>11/12 (astrocytoma)</td>
</tr>
<tr>
<td>Breast</td>
<td>0/10</td>
<td>12/12 (invasive ductal carcinoma)</td>
</tr>
<tr>
<td>Colon</td>
<td>0/10</td>
<td>11/12 (adenocarcinoma)</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>0/9</td>
<td>8/9 (sarcoma)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>0/8</td>
<td>8/8 (squamous carcinoma)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0/11</td>
<td>11/11 (clear cell carcinoma, n = 10; transitional cell carcinoma, n = 1)</td>
</tr>
<tr>
<td>Liver</td>
<td>0/8</td>
<td>Not tested</td>
</tr>
<tr>
<td>Lung</td>
<td>0/8</td>
<td>7/8 (squamous carcinoma)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>0/5</td>
<td>9/9 (non-Hodgkin's lymphoma)</td>
</tr>
<tr>
<td>Ovary</td>
<td>0/5</td>
<td>7/7 (adenocarcinoma)</td>
</tr>
<tr>
<td>Skin</td>
<td>0/6</td>
<td>6/6 (squamous carcinoma)</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0/5</td>
<td>Not tested</td>
</tr>
<tr>
<td>Stomach</td>
<td>0/10</td>
<td>9/10 (adenocarcinoma)</td>
</tr>
<tr>
<td>Testis</td>
<td>0/8</td>
<td>8/8 (malignant germ cell tumor)</td>
</tr>
<tr>
<td>Uterus</td>
<td>0/7</td>
<td>7/7 (adenocarcinoma, n = 5; malignant mixed Mullerian tumor, n = 2)</td>
</tr>
<tr>
<td>Total</td>
<td>0/130</td>
<td>122/127</td>
</tr>
</tbody>
</table>

The presence and cellular localization of CYPIB1 was investigated through immunohistochemistry with an antibody specific for CYPIB1 in different types of cancers that had developed in a variety of anatomical sites. Primary malignant tumors of these tissues constitute different histogenetic types (carcinomas, lymphomas, sarcomas, neuroepithelial tumors, and germ cell tumors), each with a different...

Fig. 3. Immunohistochemical localization of CYPIB1 in tumors and normal tissues. In tumors, CYPIB1 is specifically localized to tumor cells, and there is no CYPIB1 immuno-reactivity in normal tissues. A, normal liver; B, normal small intestine. C, normal kidney; D, carcinoma of kidney; E, normal breast; F, invasive ductal carcinoma of breast (the normal tissues in panels A, B, C, and E have been stained with hematoxylin to show histological detail). X300. Scale bar, 40 µm.
Fig. 4. Immunohistochemical localization of CYP1B1 in different types of malignant tumors and normal tissues. CYP1B1 immunoreactivity is present in tumors and absent from normal tissues. A, transitional cell carcinoma of bladder; B, normal bladder; C, high grade astrocytoma of brain; D, normal brain; E, non-small cell carcinoma of lung; F, normal lung; G, malignant lymphoma; H, normal lymph node; I, leiomyosarcoma of uterus; J, normal uterus-myometrium; K, endometrial adenocarcinoma; L, normal uterus-endometrium. ×300. Scale bar, 40 μm.

TUMOR-SPECIFIC EXPRESSION OF CYP1B1
biological behavior. These tumors also represent a range of both common and less common types of cancer. Immunohistochemistry ensures the identification of specific types of cells containing CYP1B1 and is an ideal technique for investigating the presence of CYP1B1 in tumor cells, because tumors are composed of a variable proportion of tumor cells and nontumor cells. Moreover, many tumors may only be available as formalin-fixed, wax-embedded sections.

The presence of CYP1B1 in many types of cancer suggests that this cytochrome P450 may have a crucial endogenous function in tumor cells and that CYP1B1 might also contribute to drug resistance that is observed in many types of cancer. CYP1B1 may also be important in tumor development and progression, because human CYP1B1 expressed in yeast has been shown to be capable of metabolizing a variety of putative human carcinogens (45). In addition, CYP1B1 has been shown to be capable of 4-hydroxylation of estradiol (46–48), which may be important in the development of cancers in estradiol-dependent tissues. Although CYP1B1 mRNA has been identified in a limited number of normal tissues (16, 45), our findings of an absence of detectable CYP1B1 protein in normal tissues suggest that either CYP1B1 protein is present at a very low level in normal tissues or the CYP1B1 mRNA is not translated. Recently, significantly increased 4-hydroxylation of estradiol has been identified in breast cancer (49). The expression of CYP1B1 in tumors is likely to involve several regulatory mechanisms. Regulation of the expression of forms of cytochrome P450 in liver is complex; multiple mechanisms, including transcriptional and posttranscriptional factors, are involved.

The expression of CYP1B1 in different types of malignant tumors has important consequences for both the diagnosis and treatment of cancer. New diagnostic procedures based on the presence of CYP1B1 in cancer cells can be developed, whereas the expression of CYP1B1 in tumor cells provides a molecular target for the development of new anticancer drugs that could be selectively activated by the presence of CYP1B1 in tumor cells.

REFERENCES


42. Buchmann, A., Kuhlman, W., Schwarz, M., Kunz, W., Wolf, C. R., Moll, E., Freidberg, T., and Oesch, F. Regulation and expression of four cytochrome P-450 isoenzymes, NADPH-cytochrome P-450 reductase, the glutathione transferases B and C and microsomal epoxide hydrolase in preneoplastic and neoplastic lesions in rat liver. Carcinogenesis (Lond.), 6: 513–521, 1985.


