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Minireview

Cytochrome *P450* enzymes: Novel options for cancer therapeutics

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Abstract

The concept of overexpression of individual forms of cytochrome *P450* enzymes in tumor cells is now becoming well recognized. Indeed, a growing body of research highlights the overexpression of *P450*s, particularly CYP1B1, in tumor cells as representing novel targets for anticancer therapy. The purpose of this review is to outline the novel therapeutic options and opportunities arising from both enhanced endogenous expression of cytochrome *P450* in tumors and cytochrome *P450*-mediated gene therapy. [Mol Cancer Ther. 2004;3(3):363–371]

Introduction

This commentary discusses the role and therapeutic potential of cytochrome *P450* enzymes as cancer therapeutics. Although this group of xenobiotic-metabolizing enzymes has been extensively studied in the fields of toxicology and pharmacology, their importance in cancer therapeutics is only now being realized. This review outlines the therapeutic options and opportunities arising from cytochrome *P450* expression in tumors and discusses the range of compounds currently in preclinical and clinical trials targeted at cytochrome *P450* expression in tumors. The differential expression of cytochrome *P450* enzymes within the tumor microenvironment compared with the surrounding normal tissue is also examined and the strategies used to date for the development of prodrugs, which are nontoxic to normal cells but are activated to cytotoxic agents only at the site of the tumor by individual *P450* enzymes endogenously expressed within the tumor cells, are highlighted.

In addition, current developments in *P450*-directed gene therapy, where an exogenous *P450* gene and a prodrug activated by that *P450* are delivered to the site of the tumor, are examined. As *P450*s are also capable of deactivating anticancer drugs, a brief overview of these enzymes as a mechanism of drug resistance is also discussed and the potential application of low molecular weight inhibitors to the *P450* family of enzymes is outlined. Finally, this commentary introduces the novel concept of immunotherapy targeted at tumor-specific cytochrome *P450* enzymes.

The *P450* superfamily of constitutive and inducible enzymes is responsible for the catalytic hydroxylation of a multitude of compounds with diverse chemical structures and mode of action linked only by their lipophilic nature (1). *P450*s can be categorized as either xenobiotic-metabolizing enzymes comprising CYP1, CYP2, and CYP3 with some involvement from CYP4 or as those *P450*s involved in the synthesis of endogenous substrates such as steroids, fatty acids, and prostaglandins and include CYP11, CYP17, CYP19, and CYP21 (2, 3).

The xenobiotic-metabolizing *P450* enzymes have a central role in the oxidative metabolism of a wide variety of endogenous and exogenous compounds including environmental carcinogens, plant toxins, and a wide range of anticancer drugs (4–7).

Localization and Expression of Cytochrome *P450* Enzymes in Human Tumors

While the *P450* enzymes are primarily located in the liver, there is substantial evidence that individual *P450*s are also expressed in other tissues (8–10). Of particular interest is their expression in a variety of solid tumors including breast, colon, lung, esophagus, ovarian, and soft tissue sarcomas (9, 11–20). CYP1B1 has been identified as the main CYP1 form present in a wide range of malignant tumors. Moreover, we have shown that this enzyme also demonstrates a similarly high level of enhanced expression in metastatic disease (13). This is true for abundance of mRNA and protein (11, 21–23). In contrast, CYP1B1 mRNA is expressed in a wide variety of normal tissue, but the protein is generally not detected (16, 24). Therapeutic treatment of tumors is generally targeted at the metastatic disease; thus, it is important to understand the importance and relevance of *P450*s both in primary tumor and in metastatic disease.

Recently, Dhaini *et al.* (19) demonstrated higher levels of CYP3A4/5 expression in primary osteosarcomas with

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metastatic potential using a fluorescent-based quantitative immunocytochemistry technique, highlighting this enzyme as a putative marker for metastases in osteosarcomas. CYP3A4/5 is responsible for the metabolism of several anticancer drugs including those used in the treatment of osteosarcomas (ifosfamide, vinblastine, etoposide, and doxorubicin); hence, this enzyme may also play a role in the resistance of osteosarcomas to chemotherapy.

Using a combination of real-time reverse transcription-PCR and immunoblotting, vitamin 1- α hydroxylase CYP27B1 mRNA and protein was recently shown to demonstrate increased expression in colon cancer compared with normal colon cells (25). Colon cancer is generally resistant to current anticancer drugs and this has resulted in the continued relatively poor 5-year survival (40–50%) for people with this malignancy. The enhanced expression of both CYP27B1 mRNA and protein in colon cancer may highlight an important target in the treatment of this malignancy.

The presence of *P450s* in tumor cells may be part of a pleiotropic response to tumor development (*e.g.*, *P450* enzymes may provide an essential function in tumor cells either by inactivating antitumor compounds such as 2-methoxyestradiol or by activating tumor-promoting compounds such as 4-hydroxyestradiol; Refs. 26, 27).

Anticancer Drugs Metabolized by Cytochrome *P450* Enzymes

Currently, 57 human *P450* genes and 29 pseudogenes (nonfunctional) have been identified. The sequencing of the human genome suggests that humans express fewer genes than originally estimated, so it is unlikely that many more human *P450s* remain to be identified. The diversity of cytochrome *P450* enzymes is considered to be due in part to “plant-animal warfare at the chemical level” (*i.e.*, the initial development of mammalian *P450* enzymes to overcome phytochemicals). Several of these phytochemicals are synthesized by plant *P450s* (Table 1; Refs. 28, 29). Enhanced expression in tumor cells of individual forms of these enzymes increases the potential for metabolism of anticancer drugs by the tumor cells directly. The outcome in terms of activation (*i.e.*, toxicity) or deactivation (*i.e.*, resistance) of an anticancer drug is dependent on both the relative amount and the activity of the individual *P450s* in tumor cells. In chemotherapy, it is important to establish whether *P450*-mediated metabolism of drugs is performed at the site of the tumor, out with the tumor primarily the liver, or a combination of both.

Those very toxins that evolved as plant defensive mechanisms against animal predation are now being used as valuable sources of anticancer drugs. This group of chemotherapeutic agents includes the *Vinca* alkaloids, the taxanes, and the camptothecins derived from the Madagascan periwinkle plant *Catharanthus roseus*, the Pacific yew *Taxus brevifolia*, and the Chinese tree *Camptotheca acuminata*, respectively (Table 1; Refs. 28–31).

Two other *P450s*, CYP72A1 (a secloglanin synthase) and CYP76B6 (a geraniol 10-hydroxylase), have recently been identified as major enzymes involved in the early biosynthetic reactions of the indole alkaloids from *C. roseus* (Table 1; Refs. 31, 32).

Development of Cytochrome *P450*-Based Prodrugs

Although cancer chemotherapeutics have improved over the past few decades with the introduction of several new therapies, the goal of killing cancer cells without the side effect of normal cell toxicity has yet to be achieved. The wide distribution of CYP1B1 offers tremendous opportunities for development of novel prodrugs activated by CYP1B1 only in the tumor cells. Several classes of agents, which are designed to be activated selectively by CYP1B1 to facilitate tumor-specific activation of prodrugs, are currently in preclinical evaluation.

Preclinical Evaluation

Resveratrol

The phytoestrogen resveratrol is a natural constituent of red wine and has known cancer preventative properties (33); this stilbene is metabolized by CYP1B1 to the anticancer agent piceatannol (Table 2; Ref. 34).

Aryl Oxime Prodrugs

CYP1B1 has recently undergone initial evaluation in human tumor xenografts as a target for a novel group of aryl oxime prodrugs catalyzed by this enzyme. Metabolism of these prodrugs results in the release of nitric oxide, subjecting the tumor cells to lethal doses of nitrosative stress without harming normal healthy cells (Table 2; Refs. 35, 36).

Phortress

The evolution of new anticancer drugs involves the combined forces of chemistry, pharmacy, and pharmacology. The *L*-lysylamide prodrug Phortress, a water-soluble fluorinated benzothiazole (37) derivative, is one such drug that has evolved in this manner (Table 2). This planar hydrophobic aminophenyl benzothiazole analogue is a potent agonist of the aryl hydrocarbon (Ah) receptor and switches on cytochrome *P450* CYP1A1 gene expression (38). CYP1A1 induction is regulated through the ligand-activated transcription factor, Ah receptor, and its dimerization partner, the Ah nuclear translocator; therefore, before CYP1A1 can be induced, the Ah receptor and Ah nuclear translocator need to be expressed. CYP1A1 can then be induced and convert the fluorinated prodrug Phortress into a reactive species resulting in cellular damage and subsequent apoptosis through the formation of Phortress-derived DNA adducts. Induction of CYP1A1-catalyzed biotransformation of 2-4-aminophenyl benzothiazole within tumor cells is essential for drug activation. Although members of the CYP1 family of *P450* enzymes show enhanced expression in a variety of solid tumors and anti-estrogen-resistant breast cancer cell lines (39), patients require to be screened for the presence of CYP1A1 in their tumor tissue, as not all patients may express CYP1A1 at the site of the tumor.

Table 1. Cytotoxic drugs that are metabolized by cytochrome P450 enzymes

Drug	Mechanism of action	Relative tumor activity	P450 inactivation	P450 activation	Plant source	P450 biosynthesis
Paclitaxel (7, 57)	Promotion of tubulin stabilization	Ovarian, breast, and non-small cell lung	CYP2C8, CYP3A		<i>T. brevifolia</i> (Taxaceae)	Cytochrome P450 taxane
Docetaxel (7, 57)	Promotion of tubulin stabilization	Breast and lung	CYP3A, CYP1B1		<i>T. brevifolia</i> (Taxaceae)	Cytochrome P450 taxane
Vinblastine (7, 56)	Inhibition of tubulin polymerization	Lymphoma and osteosarcoma	CYP3A		<i>C. roseus</i> (Apocynaceae)	10 β -hydroxylase CYP72A1
Vincristine (7, 56)	Inhibition of tubulin polymerization	Lymphoma	CYP3A		<i>C. roseus</i> (Apocynaceae)	secologanin synthase, CYP76B6 geraniol 10-hydroxylase CYP72A1
Vindesine (7, 56)	Inhibition of tubulin polymerization	Advanced breast and non-small cell lung	CYP3A			secologanin synthase, CYP76B6 geraniol 10-hydroxylase
Vinorelbine (7, 56)	Inhibition of tubulin polymerization	Advanced breast and non-small cell lung	CYP3A			
Ellipticine (82)	DNA intercalation and inhibition of topoisomerase II	Osteolytic breast cancer metastases	CYP3A4, CYP1A1, CYP1A2, CYP1B1, CYP2C9		Apocynaceae plants	
Etoposide (7, 83, 84)	Inhibition of topoisomerase II	Testicular small cell lung, lymphoma, and osteosarcoma	CYP3A4, CYP1A2, CYP2E1		<i>Podophyllum peltatum</i> , <i>Podophyllum emodi</i> (Berberidaceae)	
Teniposide (7, 84)	Inhibition of topoisomerase II	Small cell lung	CYP3A4/2C19		<i>P. peltatum</i> , <i>P. emodi</i> (Berberidaceae)	
Irinotecan (85)	Inhibition of topoisomerase I	Colorectal and cancers of upper gastrointestinal tract	CYP3A		<i>C. acuminata</i> (Nyssaceae)	
Topotecan (85)	Inhibition of topoisomerase I	Metastatic ovarian and small cell lung	CYP3A		<i>C. acuminata</i> (Nyssaceae)	
Dacarbazine (86)	Alkylating agent	Metastatic melanoma	CYP1A1, CYP1A2, CYP2E1			
Procarbazine (86)	Alkylating agent	Metastatic melanoma	CYP1A, CYP2B			
Mitoxantrone (57, 77)	Cytotoxic antibiotic	Breast	CYP1B1, CYP3A4			
Tamoxifen (7, 77)	Antiestrogenic	Breast	CYP3A, CYP1B1			
Cyclophosphamide (7, 86)	Alkylating agent	Sarcoma, breast, and ovarian		CYP2B6, CYP3A4		
Ifosfamide (7, 86)	Alkylating agent	Sarcoma		CYP2B6, CYP3A4		
Doxorubicin (7)	Cytotoxic antibiotic	Sarcoma, breast, and ovarian		CYP3A4		
Thiotepa (87)	Alkylating agent	Bladder		CYP2B6, CYP3A4		
Tegafur (86)	Cytostatic	Metastatic colorectal		CYP2A6		

Table 2. Novel cytochrome P450-based therapeutic options

Therapeutic	Development status	Antitumor activity	Mode of interaction with P450
Resveratrol (34)	Preclinical	<i>In vitro</i> activation by CYP1B1 to anticancer metabolite	Prodrug activated by CYP1B1
Aryl oxime (35, 36)	Preclinical	<i>In vitro</i> activation by CYP1B1 to nitric oxide resulting in cell death	Prodrug activated by CYP1B1
Phortress (38) AQ4N (45)	Phase I clinical trial Phase I clinical	Breast cancer Radioresistant tumors	Prodrug activated by CYP1A1, CYP1B1 Bioreductive prodrug activated by CYP3A4, CYP1A1, and CYP1B1
MetXia (52, 53)	Phase I/II clinical trial	Breast cancer	Retroviral vector encoding human CYP2B6 designed to activate cyclophosphamide at site of tumor
R116010 (65)	Preclinical	Hormone-independent prostate and breast tumors	Small molecule inhibitor of CYP26A
AVI-4557 (70) Zyc300 (79)	Phase I clinical trial Phase I/II clinical trial	Advanced cancer Breast, ovarian, colorectal, and prostate cancer	CYP3A4 antisense construct CYP1B1 vaccine

CYP1B1 may also be involved in the catalyzed bioactivation of Phortress; human recombinant CYP1B1 supersomes have been shown to metabolize the non-fluorinated benzothiazole precursor of Phortress (*i.e.*, DF503; Ref. 38). Indeed, benzothiazoles have been shown to modulate the expression of CYP1B1 in benzothiazole-sensitive cell lines and to increase CYP1B1 mRNA in MCF-7 cell lines (38).

In the field of toxicology and drug metabolism, induction of P450 enzymes is generally considered to be undesirable in drug discovery processes. In particular, CYP1A1 has long been known to be one of the major metabolic enzymes involved in activating many procarcinogens, the first step toward tumor formation during chemical carcinogenesis. The consequence of inducing P450s, which are not solely expressed at the site of the tumors but are also expressed in nontumor tissue, highlights potential pitfalls and unintended consequences for patients treated with cytotoxic compounds. Indeed, induction of P450s in other tissues may result in a worse prognosis for the patient due to unintentional side effects. Hence, the results of the clinical trials of Phortress are awaited with keen anticipation. A long-term follow-up of these patients is an essential requirement of any clinical trial as well as a close evaluation and monitoring of the environmental conditions under which these patients are treated.

In the future, the P450 profiling of cancer patients may result in these patients being treated with a family of drugs designed to target specific P450s under defined environmental conditions; these enzymes would have a wide spectrum of action against a range of cancers.

Bioreductive Anticancer Drugs

Unlike normal tissue, many of the cells present in the tumor are exposed to hypoxic conditions (chronic or acute lack of oxygen due to an insufficient vasculature; Ref. 40), with some cells alternating between hypoxic and aerobic

conditions (41). P450s are capable of supporting oxidative, peroxidative, and reductive metabolism of a range of xenobiotic and endogenous compounds including the generation of active arachidonic acid metabolites (42). Under a hypoxic environment, P450 enzymes may function as reductive enzymes.

The potential of such approaches has already been shown with the use of the bioreductive drugs designed to be activated in the hypoxic microenvironment of the tumor by individual P450s. Bioreductive drugs are designed to target treatment-resistant hypoxic tumor cells. Radiation damage to hypoxic tumor cells is compromised by lack of oxygen; thus, one of the major uses of bioreductive drugs is as an adjunct to radiotherapy (43, 44).

It is well established that the topoisomerases I and II inhibitor AQ4N, an alkylaminoanthraquinone *N*-oxide (1,4-bis{[2-(dimethylamino-*N*-oxide)ethyl]amino}5,8-dihydroxyanthracene-9,10-dione), is a relatively nontoxic prodrug that is activated through enzymatic reduction selectively under hypoxic conditions to the corresponding basic amine (45). CYP3A4, CYP1A1, and CYP1B1 all contribute to AQ4N's bioreduction. This biotransformation introduces a cationic charge, which can greatly increase the DNA binding affinity, providing a hypoxia-selective prodrug activation mechanism. Thus, under hypoxic conditions, AQ4N can be reduced to a positively charged stable compound AQ4, which possesses marked affinity for DNA and the ability to inhibit topoisomerase II. Moreover, AQ4 is able to permeate the surrounding tumor cells, eliciting a "bystander" killing effect (45). AQ4 is 1000-fold more cytotoxic than AQ4N, which shows only weak DNA binding and limited ability to inhibit topoisomerase II (46).

AQ4N has also recently been validated for use in gene-directed prodrug therapy (GDEPT; Ref. 47) in RIF-1 murine tumor cells transfected with a mammalian expression vector pcDNA 3.1 containing CYP3A4. The authors demonstrated that the presence of CYP3A4 increased the metabolism of AQ4N and significantly

increased the killing power of this cytotoxin. AQ4N is the first P450-activated bioreductive drug in clinical trials and the results from the phase I clinical trials for esophageal cancer are expected this year.³

Gene Therapy: Targeting Drug and Enzyme to the Tumor

Another therapeutic approach currently being investigated is GDEPT. This involves targeting of prodrugs and the genes for exogenous enzymes (*i.e.*, P450s in the context of this review) to tumor cells. This results in tumor-specific conversion to active drugs. GDEPT has demonstrated selectivity and efficacy in experimental systems as well as clinical trials. However, several issues require to be addressed before this strategy can become routine practice in a clinical setting. The DNA vectors used in gene therapy should be characterized by maximum efficiency and minimal toxicity. These "magic bullets" should be targeted, protected from degradation and immune attack, and safe for the recipient and the environment (*i.e.*, replication deficient, low potential for viral integration, and insertional mutagenesis). Moreover, the therapeutic gene should be appropriately regulated and expressed only for as long as required to initiate an appropriate "bystander" effect. Vehicles such as retroviruses and adenoviruses have been adopted in clinical studies with varying results. New therapeutic modalities are also being explored to overcome the limitation of poor gene transfer and patient toxicity, including bacteria, adeno-associated and herpes simplex viruses, lentiviruses, cationic polymer-DNA complexes, and electroporation.

A major concern in gene therapy is the inability to kill 100% of the tumor cells. GDEPT provides a mechanism to overcome this problem by combining an exogenous enzyme with prodrugs such as cyclophosphamide and ifosfamide, which elicit a strong "bystander" effect, killing the surrounding cells even those that do not express the prodrug-activating enzyme.

P450-based GDEPT strategies were initially explored to overcome the low levels of active P450 in the brain and poor penetration of the cytotoxic compound 4-hydroxycyclophosphamide across the blood-brain barrier. Historically, this strategy used the rat CYP2B1 to activate oxazaphosphorines. Initial studies demonstrated that CYP2B1 could be transfected into rat C6 glioma cells, rendering them chemosensitive to the parent prodrug cyclophosphamide (48). The glioma cells were then inoculated stereotactically into the brains of athymic mice treated with fibroblasts transfected with a retrovirus encoding CYP2B1. Administration of cyclophosphamide either intrathecally or intratumorally prevented meningeal tumor spread and reduced the size of tumors in these animals (48). CYP2B1 can sensitize tumor cells to the cytotoxic action of the alkylating prodrugs such as cyclophosphamide and ifosfamide that

are normally activated to 4-hydroxylated metabolites in the liver (49). These metabolites spontaneously decay to phosphoramidate mustard and acrolein that alkylate proteins and DNA, respectively. The short half-life of these metabolites makes this an ideal approach for targeting drugs to tumor cells and avoiding any possible diffusion of toxic products to normal nontumor tissue.

In a separate study, replication-deficient retroviral and adenoviral-mediated gene transfer of CYP2B1 into 9L glioma cells significantly increased the sensitivity of these tumor cells to the cytotoxic effects of cyclophosphamide. In addition, in Fischer 344 rats with intracerebrally implanted 9L tumors, a modest increase in survival was observed when treated *in vivo* with cyclophosphamide. Similar results were obtained with either the retrovirus or the adenovirus packaged cell lines (50).

Studies on transfected human-derived tumor cell lines with CYP2B1 encoding plasmids rendered these cells susceptible to the cytotoxic effects of cyclophosphamide. Moreover, the sensitivity to cyclophosphamide was maintained in culture, even when only a fraction of the cells expressed P450 (51).

A phase I/II trial of patients ($n = 14$) with inoperable pancreatic cancer who had CYP2B1 delivered to the tumor vasculature (in microencapsulated genetically modified allogenic human 293 embryonic kidney cells) showed a favorable reduction in tumor size compared with a control group receiving gemcitabine (49). Although these studies are promising, the incorporation of a rodent gene into a human patient may elicit an unwanted immunological response in some patients, which could prejudice therapy.

To overcome a potential immune response that could be detrimental to the patient, the human CYP2B6 gene has replaced the rat CYP2B1 gene in a new construct. MetXia, a novel third-generation retroviral vector encoding the human CYP2B6 gene (52, 53) and designed to be injected directly into the site of the tumor, has recently shown promising interim results in a phase I/II trial in advanced breast cancer resolving the concerns over safety and gene transfer. Furthermore, several patients treated with this product exhibited a reduction in the size of the tumor.

Novel strategies are being used to further enhance the specificity of P450-driven GDEPT. Activated macrophages have recently been used as "silent carriers" of gene therapy. These cells that form a significant portion of the tumor mass were transduced with a hypoxia-regulated adenoviral vector and used to deliver a CYP2B6 gene to the hypoxic region of tumors (54).

One of the major challenges of GDEPT is to ensure that the gene is expressed for as long as is required in the tumor cell to initiate a prolonged "bystander" kill. Tumor cells infected with the viral vector delivering CYP2B6 and cyclophosphamide are more sensitive to the activated drug than P450-deficient tumor cells. This problem was recently addressed by transducing tumor cells stably transfected with CYP2B6 with a retroviral vector encoding the antiapoptotic factor p35. By delaying but not preventing cell death in the P450-expressing tumor cells, p35 substantially prolonged the

³ KuDOS Pharmaceuticals Web site provides detailed information in the current status of AQ4N (http://www.kudospharma.co.uk/r_d/aq4n.php; accessed September 9, 2003).

activation of cyclophosphamide by the "P450 factory cells" without inducing drug resistance in the P450 tumor-expressing cells, resulting in increased "bystander" cytotoxicity to the surrounding P450-deficient cells (55).

The current advances in P450 GDEPT, using human P450 enzymes to avoid undesirable immune responses and employing safer delivery systems such as microencapsulation and direct retroviral delivery systems, highlight the potential of these novel tumor-directed therapeutics.

Cytochrome P450 Enzymes as a Mechanism of Drug Resistance

Paradoxically, the presence of functionally active P450 enzymes in tumor cells may also have a negative effect on chemotherapy-mediated tumor cell death through the deactivation of a given anticancer drug. Indeed, the importance of P450-mediated metabolism of anticancer drugs has recently been highlighted by Yao *et al.* (56) who have shown that CYP3A may play a role in drug resistance to the *Vinca* alkaloids and by Dhani *et al.* (19) who implicate CYP3A4/5 as playing a role in the resistance of osteosarcomas to chemotherapy.

Paclitaxel, a diterpenoid taxane licensed for use as first-line therapy for breast, ovarian, and small cell lung cancer, undergoes hydroxylation by CYP2C8 to a 6-hydroxymetabolite 30-fold less toxic than the parent compound. A related taxane, docetaxel, is metabolized to pharmacologically less active compounds by CYP3A4/5 (Table 1; Ref. 57). A recent study has shown that the taxanes appear to be capable of inducing their own degradation through the induction of CYP3A and CYP2C8 (58, 59). However, Nallani *et al.* (60) recently showed that the docetaxel-mediated increase in CYP3A activity and enzyme levels was significantly lower than for those mediated by paclitaxel. Synold *et al.* demonstrated that enhanced expression of P450 by the taxanes was through activation of the xenobiotic receptor SXR (*i.e.*, the taxanes are ligands for SXR). This protein has a central role in the regulation of the P450 enzymes CYP3A4 and CYP2C8 through transcriptional activation (61). The findings that the taxanes may be regulating their own metabolism have important clinical implications.

Small Molecule Inhibitors to Cytochrome P450s

CYP24 and CYP26A, which are involved in the metabolism of vitamins A and D, two vitamins considered to have a role in cancer prevention, also demonstrate enhanced expression in tumor cells. Indeed, CYP24 has recently been implicated as an oncogene (62). This P450 is involved in the degradation of the antitumorigenic agent 1,25-dihydroxyvitamin D3 (1,25-D3) used in the therapy of malignant diseases such as prostate cancer (63). The CYP24 gene has shown enhanced expression due to amplification of gene copy number, resulting in increased expression of mRNA and protein activity in breast cancer; some prostatic cancer cells and several lung cancer cell lines have also been found to express high levels of CYP24 (63). Genistein, a widely occurring isoflavonoid, inhibits cyto-

chrome enzymes and also exerts antitumorigenic effects. Recent studies, in conjunction with the histone deacetylases inhibitor trichostatin A, demonstrated that genistein resensitized prostate cancer cells to the antitumor effects of 1,25-D3; the addition of trichostatin A appeared to prevent the transcriptional inhibition of CYP27B1, which synthesizes 1,25-D3 (64).

Vitamin A (retinoic acid) and its synthetic analogues (retinoids) can induce the differentiation of several tumor-derived cell lines, which have also been shown to inhibit the growth of solid tumors. The human retinoic acid 4-hydroxylase CYP26A (65) is implicated in clinically acquired resistance to retinoic acid as cells overexpressing this P450 escape the antitumorigenic role of retinoic acid. Certain tumor cell types including prostate, non-small cell lung carcinomas, breast adenocarcinomas, and acute promyelocytic leukemia appear to express CYP26A at constitutively high levels (66–69). Therefore, inhibitors of CYP26A may represent a novel class of anticancer drugs and potentially provide exciting therapeutic options for patients treated with retinoid therapy. Indeed, R116010, a retinoic acid metabolism blocking agent developed by researchers at Jansen Research Foundation (Belgium), is a selective CYP26A inhibitor with strong antitumor activity against androgen-independent rat prostate and estrogen-independent mouse mammary tumors (Table 2; Ref. 65).

Antisense-Based P450 Therapy

A new antisense phosphorodiamidate morpholino oligomer drug AVI-4557 that specifically targets cytochrome P450 CYP3A4 (70) has recently completed phase I studies with phase II studies planned for later this year. As CYP3A4 is involved in the deactivation of several cytotoxic agents including the taxanes and the *Vinca* alkaloids (71, 72), regulating expression of CYP3A4 at the site of the tumor holds great promise for increasing the cytotoxic efficacy of these drugs. However, the use of antisense technology against CYP3A4 must be taken with a degree of caution because unlike CYP1B1, which is regarded as a tumor-specific enzyme, CYP3A4 is one of the major cytochrome P450 enzymes expressed in the human liver and is responsible for the metabolism of more than 50% of all the drugs currently in use (73–75).

CYP1B1 is a tumor-selective P450, which has been shown to interact with several anticancer drugs including the taxanes. Therefore, this P450 is a potential mechanism of tumor-specific anticancer drug inactivation (76, 77). Recently, Bournique and Lemarie (78) demonstrated that docetaxel binds to CYP1B1 and acts as an effector of this enzyme.

Immune-Based Therapy

The specific localization of individual P450s, particularly CYP1B1, in tumor cells can also be exploited in immune-based therapy. As CYP1B1 is overexpressed in a tumor cells, it represents an ideal target for immunotherapy. Pre-clinical trials of a CYP1B1 DNA vaccine Zyc300 have been

shown to induce a specific T-cell response to destroy cancer cells. Phase I/IIa clinical trials are currently under way with this vaccine, and to date, the vaccine has produced promising result (79).

Influence of Cytochrome P450 Polymorphisms on Drug Development

Although there are a considerable number of studies on P450 polymorphisms and tumorigenesis, few of these focus on P450 polymorphisms in response to therapy. There is accumulating evidence that P450 polymorphisms contribute significantly to interindividual and interethnic variations in the capacity of individuals to metabolize chemotherapeutic drugs. This variation in metabolism may have a major bearing on the response of the patient to chemotherapeutic intervention (80).

Development of novel P450 therapeutics must take into account the major P450 variants, which may influence patient response to treatment. The impact of P450 polymorphisms on drug metabolism can be shown by the highly polymorphic CYP2D6, a major drug-metabolizing enzyme, with 13 copies of the gene in some ultrafast metabolizers (80). CYP3A4, the most highly abundant form of P450 in adults, demonstrates wide interindividual variation, which may have an important influence on drug response. Single base changes (single nucleotide polymorphisms) in the coding region of CYP3A4 may alter the activity of this P450. Indeed, Dai *et al.* (81) recently demonstrated by direct sequencing of genomic DNA 28 single nucleotide polymorphisms in the CYP3A4 gene, 5 of these were in the coding region that may increase or decrease CYP3A enzyme activity (81).

Hence, prior to administration of P450-activated drugs or inhibitors, P450 profiling of patients for expression and gene copy number would help to predict response to therapeutic treatment.

Conclusions

A major objective of cancer research is the development of therapeutic agents specifically aimed at tumor cells and eliminating or minimizing systemic toxicity. The P450 group of enzymes represents such a therapeutic target.

Although several P450s (CYP1A, CYP2C, and CYP3A) exhibit enhanced expression in tumor cells, several of these also display considerable expression in normal tissue including the liver. However, the environmental conditions (*i.e.*, hypoxia) of the tumor differ compared with those observed in normal tissue, enabling these enzymes to differentially metabolize particular drugs (*e.g.*, AQ4N) to cytotoxic compounds only in tumor cells. Thus, individual P450s, which are overexpressed in tumor cells, may represent exciting and novel anticancer targets by providing P450-mediated metabolism at the site of the tumor (*i.e.*, the site of anticancer drug action). Moreover, by identifying the patient's complement of functionally active cytochrome

P450s within the tumor and nontumor tissues, it will be possible to tailor patient-specific therapeutic regimens including prodrugs, clinical inhibitors, and immunotherapy to improve the treatment of a variety of human tumors.

Useful Websites

<http://drnelson.utmem.edu/CytochromeP450.html> [Detailed information on the classification and nomenclature of cytochrome P450s.]

http://www.gentest.com/human_p450_database/index.html [Excellent database for information on P450 drug metabolism and toxicity.]

<http://www.icgeb.org/~p450srv/> [Database providing detailed information for all P450 enzymes.]

<http://medicine.iupui.edu/flockhart/> [Useful website for providing detailed information on clinically used drugs metabolized by cytochrome P450s.]

<http://www.zycos.com> [Information on Zycos biotechnology phase I/IIa clinical trials of CYP1B1 DNA vaccine Zyc300.]

http://www.kudospharma.co.uk/r_d/aq4n.php [Information on KuDOS Pharmaceuticals phase I clinical trial of AQ4N.]

Search Criteria

Reference criteria were identified by searching of PubMed and Science Citation Index. The search terms used were cytochrome P450; CYP1B1, anticancer; chemotherapeutic; drug; and plant. These terms were combined with the words tumor and neoplasm. Further references were selected from retrieved articles, which provided additional information or were of personal interest.

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