Cytochrome P450 1B1: a novel anticancer therapeutic target

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Cytochrome P450 (CYP)1B1 is overexpressed in tumor cells and is also recognized as a biomarker of the tumor phenotype. This review highlights the tremendous potential of this enzyme as a novel cancer therapeutic target. The range of therapeutic strategies including immunotherapeutics, CYP1B1-activated prodrugs and CYP1B1 inhibitors, that are currently being developed to exploit the presence and activity of CYP1B1 in tumor cells is outlined. The therapeutic strategy, which is at the most advanced stage of development, is a CYP1B1-based vaccine which has already successfully completed a Phase I clinical trial.

Over the past 50 years, major advances in cancer research have been achieved with the advent of novel therapeutic regimens for more patient-specific therapies. However, the dual goals of tumor-selective targeting while minimizing normal cell toxicity have yet to be achieved.

The purpose of this review is to highlight cytochrome P450 (CYP)1B1 as a novel therapeutic target and outline the variety of pharmacologic and biologic options that are being developed to exploit this molecule as an effective anticancer drug target.

Evidence for enhanced expression of CYP1B1 in tumor cells *in vivo* & *in vitro*

The CYP superfamily of hemoproteins are involved in the oxidative metabolism of a wide range of toxic foreign compounds (xenobiotics), and have a central role in influencing the response of established tumors to anticancer drugs; these enzymes can either activate or deactivate many anticancer drugs. The outcome, in terms of drug activation (i.e., results in cytotoxicity) or deactivation (i.e., no cytotoxicity and potentially resistance), is dependent upon the relative amount and activity of specific CYPs in individual tumor cells.

The authors' research has been paramount in establishing the concept of enhanced expression of CYPs, in particular, those belonging to the CYP1 family, in a variety of different histological types of tumors [1–4].

The authors have identified CYP1B1 as the main CYP present in a wide range of human cancers of different histological types. This CYP is overexpressed in these tumors [1,3,5] and is specifically localized to tumor cells [1,5–7]. Moreover, the authors have shown that this enzyme also demonstrates a similarly high level of enhanced expression in metastatic malignant disease [8]. Enhanced expression of CYP1B1 has also been independently detected in a number of malignant tissues, tumor-derived and transformed cell lines, including prostate cancer cells [9]; lung carcinoma cells [10]; transformed B-lymphocytes [11] and immortalized oral keratinocytes [12]. CYP1B1 is now widely regarded as a biomarker of the neoplastic phenotype. In contrast, although CYP1B1 mRNA appears to be expressed in a wide variety of normal tissues, the corresponding CYP1B1 protein has generally not been detected [1,6,13].

CYP1B1 in renal cell cancer

Recently, the authors have demonstrated the presence of metabolically active CYP1B1 in renal cell carcinoma, which is the most common malignancy of the adult kidney and has a poor prognosis due to its late presentation and frequent resistance to current anticancer drugs. The presence of metabolically active CYP1B1 in tumor cells represents a potential therapeutic target in this malignancy [14]. Measuring CYP1B1 activity was achieved by assaying resorufin production from the *O*-deethylation of ethoxyresorufin in the presence or absence of the CYP1 inhibitor α-naphthoflavone. The key finding of this study was the presence of metabolically active CYP1B1 in 70% of renal cell carcinomas with no CYP1B1 activity in normal kidney [14]. The presence of active CYP1B1, which is overexpressed in tumors and which is capable of metabolizing anticancer drugs within the tumor cells, offers tremendous opportunities for the development of novel prodrugs activated by CYP1B1 only in the tumor cells [6,7].

CYP1B1 as a therapeutic target

The authors' finding that CYP1B1 protein is overexpressed in a wide range of human cancers of different histogenetic types, but is either expressed at low level or is absent in non-neoplastic tissues, was the subject of a patent application in 1997 [PCT;

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future medicine

Table 1. CYP1B1-based patent applications.

PCT	Scope of patent application	
CYP1B1 as a therapeutic target		
WO9712246[§]	Diagnostic and therapeutic targeting of CYP1B1	
WO0056773	Antibodies specific for CYP1B1	
CYP1B1 proprodrug activation		
WO02067930	Benz-indole and benzo-quinoline derivatives as prodrugs for tumor treatment	Prodrug analogs of duocarmycin
WO02068412	Pyrrolo-indole and pyrrolo-quinoline derivatives as prodrugs for tumor treatment	Prodrug analogs of duocarmycin
WO9940056	Hydroxylation activated prodrugs	The prodrug moiety may have a steroid carbon carrier framework i.e. estradiol
WO9940944	Hydroxylation activated drug release	
CYP1B1 inhibitors		
WO0158444	CYP1B1 inhibitor co-therapy	
WO03029176	4-C2-6alkoxy-substituted chalcones as therapeutic agents	
WO03018013	Stilbene derivative having cytochrome P450 1B1 inhibitory activity, pharmaceutically acceptable salt thereof, method for preparing the same and composition including the same	
CYP1B1-based immunotherapy		
WO0242325	CYP1B1 nucleic acids and methods of use	Describes the development of CYP1B1-based vaccines

[§] Key patent application which describes the potential uses of CYP1B1 in tumor diagnosis and therapy.
CYP1B1: Cytochrome P450.

WO9712246] and a US patent has subsequently been issued [US 6242203]. This discovery has subsequently been followed by the generation of a highly specific monoclonal antibody against CYP1B1 [5], which can potentially be used in the diagnosis or treatment of cancers that show enhanced CYP1B1 expression. An increasing number of patent applications exploiting the therapeutic potential of CYP1B1 as an anticancer target have been made in the last 5 years (Table 1). Indeed, several therapeutic strategies based on the overexpression and activity of CYP1B1 in tumors are currently in different stages of preclinical and early clinical development and will be outlined. These approaches include CYP1B1 based immunotherapeutics, CYP1B1-mediated prodrug activation and both low-molecular-weight chemical and nucleic acid-based antisense inhibitors of CYP1B1.

CYP1B1-based immunotherapy

The importance of CYP1B1 in tumors as an anticancer target is currently best highlighted as a tumor antigen. ZYC300 is a CYP1B1-based DNA vaccine [15], designed to stimulate the immune system against tumor cells expressing CYP1B1 and it has been shown to promote high reactivity to CYP1B1 producing a strong $CD8^+$ T-cell response [15,16]. The ZYC300 DNA vaccine elicits a polyclonal T-cell response; one of the activated T-cell populations recognizes the CYP190 peptide in the context of HLA-A2 on the surface of tumor cells [16]. A Phase I trial of ZYC300 at the Dana Farber Cancer Institute of Harvard Medical School, USA, was recently completed in a cohort of late stage cancer patients. The vaccine was well tolerated, and it was observed that the number of vaccine doses administered correlated with a decrease in disease progression. Moreover, a significant improvement in survival was observed in those patients who produced an immune response to the CYP1B1 therapy. Indeed, somewhat surprisingly, at least one patient has remained stable and required no further therapy for 2 years following entry into this Phase I study. On completion of ZYC300 treatment, all other patients went on to receive salvage therapy. In general, patients who did not respond to ZYC300 had no clinically significant response following salvage; whereas, ZYC300 responders showed clinical benefit following salvage, including patients with stable

disease, partial and complete responses. Follow-up Phase II trials exploring the efficacy of ZYC300 are currently being planned [101].

CYP1B1-mediated prodrug activation

CYP1B1 is now widely acknowledged as a highly promising target for prodrug-activated anticancer therapy as it provides the opportunity for selective prodrug activation to occur in tumor cells without toxicity in normal cells [7,17]. Indeed, CYP1B1 has undergone initial evaluation in human tumor xenografts as a target for a novel group of aryl oxime prodrugs catalyzed by this enzyme. Bioactivation of these prodrugs results in the release of nitric oxide, subjecting the tumor cells to lethal doses of nitrosative stress without harming healthy cells [18,102].

Prodrugs under development

A range of pyrrolo-indole and pyrrolo-quinoline derivatives have been patented as prodrugs and are currently under preclinical investigation as targets for aromatic oxidation (e.g., hydroxylation) by CYP1B1 intratumorally (Table 1), resulting in the release of activated DNA alkylating agents which would cause cell death or adversely effect the tumor cells. Several novel therapeutic strategies utilizing a range of compounds as prodrugs, including isoflavonoids and stilbenes, targeted at CYP1B1 have been the subject of patent applications and are currently in preclinical development (Table 1). It is anticipated that one or more of these compounds will enter clinical trials in the near future.

The use of CYP1B1 inhibitors to overcome anticancer drug resistance

Drug resistance is one of the major problems in the treatment of cancer. A range of pharmacological mechanisms combine to result in a poorer response to chemotherapy and decreased patient survival.

A greater understanding of the factors involved in anticancer drug resistance would be of considerable benefit. The authors have previously demonstrated that CYP1B1 metabolically inactivates a range of structurally diverse anticancer drugs (docetaxel, doxorubicin, paclitaxel, mitoxantrone and tamoxifen) and as a consequence of this, CYP1B1 also mediates anticancer drug resistance [4,8,19]. In those patients with ovarian cancers and who had received docetaxel there was poorer survival in patients whose tumors showed a high level of CYP1B1 expression [8]. Indeed, Bournique and Lemarie have confirmed our findings that CYP1B1 interacts with docetaxel [20]. The authors' *in vitro* studies have also previously highlighted the use of specific low-molecular-weight chemical inhibitors to CYP1B1 in modulating the cytotoxic profile of a range of structurally diverse anticancer drugs with CYP1B1 [4,19]. However, there are currently no generally applicable and validated animal models for studying CYP1B1 activity in tumors and the development and validation of appropriate models represents a considerable challenge.

Small molecule inhibitors of CYP1B1

CYP1B1 has been shown to catalyze the *O*-demethylation of biochanin A (a principle isoflavonoid found in red clover) to produce genistein [21]. This widely occurring isoflavonoid, has recently been shown to inhibit several carcinogenic signaling pathways through reduced gene expression of *CYP1B1*, *EGFR* (epidermal growth factor-receptor), *egr-1*, *AKT2* (protein kinase B), *NELL2* and *DNA ligase III* [22]. It has been demonstrated that tetramethyl stilbene inhibited the catalytic activity (inhibition constant [Ki] = 3 nM; inhibitory concentration of 50% [IC_{50}] = 6 nM) of *CYP1B1* [23–25]. A methylated derivative of oxyresveratrol which is a potent and selective inhibitor of CYP1B1 has also recently been developed [26]. The ability to inhibit CYP1B1, resulting in increased efficacy of chemotherapeutic intervention may have important clinical implications and provide a mechanism by which the efficacy of the current generation of cytotoxic anticancer drugs can be enhanced.

Antisense-based therapy

Molecular therapeutics such as antisense-based CYP strategies represent a further option for inhibiting the metabolism of anticancer drugs at their target site. By suppressing or preventing CYP1B1 expression at the site of the tumor it should be possible to increase the efficacy of clinically important anticancer drugs, which interact with and are inactivated by CYP1B1 in tumor cells [19].

Indeed, an antisense phosphorodiamidate morpholino oligomer drug AVI-4557 targeted at another CYP; CYP3A4 [27], has recently completed a proof-of-principle Phase I trial in healthy volunteers [103] showing that the antisense approach can reduce CYP activity. CYP3A4 is involved in the metabolism of a wide variety of anticancer drugs and by blocking the expression of this CYP the effectiveness of a range of anticancer drugs could potentially be enhanced.

The success of this antisense-based therapy provides proof-of-concept for the use of antisense constructs to suppress the expression of *CYP1B1*. Moreover, as *CYP1B1* is overexpressed in tumor

cells it provides an excellent target for antisense technology to enhance anticancer drug-associated cytotoxicity at the site of the tumor.

Initial studies evaluating the efficacy of CYP1B1 inhibitors

McFadyen and colleagues previously evaluated the metabolism of a variety of anticancer drugs by human CYP1B1 expressed in the V79 Chinese hamster ovary cells [4], in addition to a competitive assay system which employs microsomes isolated from lymphoblastoid cells genetically engineered to express human CYP1B1 [4,19]. From the authors' previous *in vitro* studies it has been demonstrated that the presence of CYP1B1 increases the resistance of cells to the cytotoxic effects of anticancer drugs [4,8], thereby establishing a rationale for the use of CYP1B1 inhibitor therapy in combination with chemo-therapeutic intervention. The authors' ongoing studies [Unpublished Data] have assessed an extensive range of anticancer drugs to determine their interaction with CYP1B1; the initial findings show that several of these cytotoxic anticancer drugs act in a substrate-dependent manner. According to an earlier study conducted by the authors, the evaluated efficacy of inhibiting CYP1B1 on the cytotoxic profile of anticancer drugs has demonstrated interaction with CYP [4]. These findings support the authors' concept that combining anticancer drugs with CYP1B1 inhibition will increase the efficacy of those drugs that are inactivated by CYP1B1.

Although small molecule inhibitors may be useful in reducing the activity of CYP1B1, development of a specific small molecule enzyme inhibitor that occupies the 3D active site of the enzyme, without also affecting closely related CYP is a difficult and arduous task. Antisense technology has made it much more straightforward to specifically silence a particular enzyme by designing a polynucleotide construct that binds to the target gene mRNA and blocks expression of functional protein. Since greater than 90% of any mRNA sequence is involved in or shielded by intramolecular interactions and is not available to base pair with an antisense oligonucleotide, the key to the design of an effective antisense construct is the identification of accessible regions of the target transcript.

Future perspective

Our knowledge and understanding of tumor-selective CYP expression has increased considerably over the past decade with an increasing variety of novel therapeutic strategies being developed to exploit the enhanced expression of these enzymes, in particular CYP1B1, in tumor cells. However, if we are to successfully target the expression of individual CYP in tumor cells, it is important to define the profile of individual tumors and identify those CYPs that are overexpressed [28]. The prognostic and predictive significance of CYP expression in malignant tumors also needs to be defined.

The advent of novel technologies such as tissue microarrays and protein chips as a routine diagnostic tool during the next decade should facilitate the potential of tailoring patient-specific therapeutic regimens based on individual CYP expression in tumor cells.

Executive summary

***Evidence for enhanced expression of CYP1B1 in tumor cells* in vivo *and* in vitro**

- CYP1B1 is selectively overexpressed in tumor cells. CYP1B1 is a biomarker of the tumor phenotype.

CYP1B1 in renal cell cancer

- CYP1B1 is overexpressed and is metabolically active in cancer of the kidney.

CYP1B1 as a therapeutic target

- Several anticancer therapeutic strategies based on targeting CYP1B1 are in preclinical and early clinical development. These approaches include CYP1B1 vaccines, CYP1B1-based prodrug activation and inhibitors of CYP1B1.

CYP1B1-based immunotherapy

- A CYP1B1 vaccine has been developed.
- This vaccine has successfully completed a Phase I clinical trial and Phase II trials are planned.

CYP1B1-mediated prodrug activation

- A CYP1B1 based prodrug activation strategy has the greatest potential to offer significant therapeutic benefits whilst overcoming the challenges of dose-limiting toxicity that limit the use of many of the current generation of anticancer drugs.

The use of CYP1B1 inhibitors to overcome anticancer drug resistance

- The use of inhibitors of CYP1B1 as co-therapy has the potential to enhance the efficacy of both current and novel anticancer drugs.
- Both chemical inhibitors and antisense approaches to inhibiting CYP1B1 are being developed.

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