



## MANIPULATING CYTOCHROME P450 ENZYMES: NEW PERSPECTIVES FOR CANCER TREATMENT

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### ABSTRACT

*Increasing drug efficiency and reducing drug overall toxicity are two of the main goals of today's drug chemotherapy of cancer. In general this could be achieved by searching new ways for selective active drug accumulation in cancer cells by manipulating local drug metabolism or delivery. In this short review, on the basis of the main directions described by McFadyen et al. (Mol Cancer Ther 2004; 3(3): 363-371), new data is reported for localization and expression of cytochrome P450 enzymes in human tumors, development of cytochrome P450-based and gene-directed enzyme activated prodrugs, antisense-based P450 and immune-based therapy, cytochrome P450 polymorphism in development of anticancer drugs. New discoveries of molecular biology of cancer give us hope for more successful development of modern cancer chemotherapy. **Biomed Rev 2017; 28: 120-124.***

**Keywords:** cytochrome P450 isoforms, cancer cells, drug metabolism, drug delivery

### INTRODUCTION

The cytochrome P450 superfamily of hemoproteins are involved in the oxidative metabolism of a wide range of toxic foreign compounds 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 achievements of the various "omics" scientific trends enable us more successfully to increase efficiency and reduce the toxicity of anticancer agents by selective manipulation of the expression and activity of different cytochrome P450s in tumor cells (2).

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

A recent comprehensive overview showed expression of practically all the cytochrome P450 superfamily in human

bronchial and peripheral lung tissue with high inter-individual variety of expression in non-small lung cancer cells which in turn could explained the different individual susceptibility towards the deleterious effects of inhaled chemical toxicants and carcinogens (3). A well-known fact is the presence of metabolically active *CYP1B1* in 70% of renal cell carcinomas with no *CYP1B1* activity in normal kidneys (4). Over expression of *CYP1B1* and other CYP's isoforms were found also in tumors of the lung, breast, liver, gastrointestinal tract, prostate and bladder which turns it into a crucial factor in cancerogenesis as well as in an important target for successful anticancer therapy (5-11). This makes *CYP1B1* an early-stage tumor marker (12). *CYP2W1* has also been identified as having tumor-specific expression in colon and adrenal cancer (13) and the extent of this expression could serve as an independent prognostic factor for overall survival (14). Elevated *CYP1A1* expression was shown in human bladder cancer relative to normal human tissues (15). *CYP2J2* was found to be overexpressed in adenocarcinoma and breast carcinoma as well as in hematopoietic system malignant cells (16).

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**Table 1.** Cytochrome P450' isoforms specific metabolism of different anticancer drugs

Cytochrome P450's	Drugs	Deactivation	Activation
CYP1A2	Dacarbazine	no	yes
CYP2A6	Ftorafur, Tegafur	no	yes
CYP2B	Altretamine	yes	no
	Cyclophosphamide, Ifosfamide, Procarbazine, Thiotepa	no	yes
CYP2C	Bexarotene, Paclitaxel, Tretinoin	yes	no
CYP2D6	Doxorubicin	yes	-
	Idarubicin	no	yes
CYP3A4	Busulfan, Cytarabine, Gefitinib, Irinotecan, Teniposide, Topotecan, Vinblastine, Paclitaxel	yes	no
	Ifosfamide, Ellipticine, MMDX	no	yes

CYP4Z1 is overexpressed in breast carcinoma (3). All recent data showed that the CYP expression profile in an individual tumour is likely to be an important determinant in predicting the outcome of cancer chemotherapy (17).

#### Anticancer Drugs Metabolized by Cytochrome P450 Enzymes

Several anticancer agents are metabolized by one or more cytochrome P450s with two different outcomes of the corresponding metabolites: deactivation or activation (18, 19) (Table 1). A specific CYP450 Reductase-mediated doxorubicin reduction under anaerobic conditions was found.

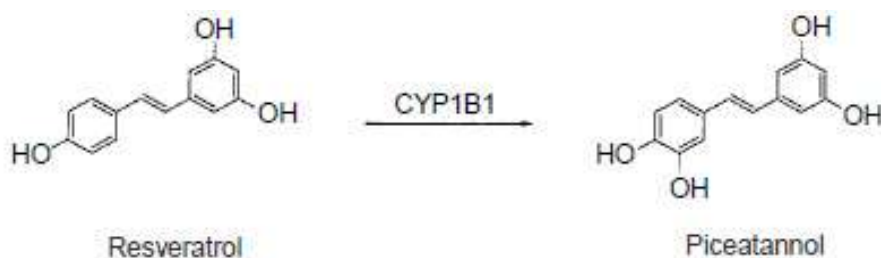
#### Development of Cytochrome P450-Based Activated Prodrugs

The specific preferential expression of different cytochrome P450 isoforms in human tumor cells allows the synthesis of selectively activated prodrugs (20), like:

- Anticancer nitric oxide donors – aryl oximes, N-nitrosamines, N-hydroxyguanidines (21).
- Phortress (dihydrochloride salt of 2-(4-amino-3-methyl-2-(4-amino-3-methyl-phenyl)-5-fluorobenzothiazole selectively) after sequestration only in benzothiazole sensitive (MCF-7 breast) cells and binding to cytosolic aryl hydrocarbon receptor (AhR), proceeds with translocation of the complex to the cell nucleus where the consistent activation of CYP1A1 gene transcription leads to subsequent induction of CYP1A1-catalyzed metabolism of Phortress to reactive electrophilic species and results in DNA-adduct

formation. Phortress is active against ovarian, breast and colorectal cancer (22-24).

- Aminoflavone exerts Phortress-like cytotoxic action after CYP 1A1 and 1A2 induction (2).
- DUM-135 is activated by CYP1B1 to form its active metabolite, DMU-117 (a tyrosine kinase inhibitor) (2).
- Methoxymorpholinyl doxorubicin (MMDX) is a novel pro-drug activated by human CYP3A4 to a potent long-lived and cell-permeable cytotoxic metabolite (25).
- Resveratrol is activated by CYP1B1 to an active metabolite Piceatannol, a tyrosine kinase inhibitor with antileukaemic activity (scheme 1) (26, 27).



- Pro-drug analogs of duocarmycin were activated by CYP1B1 (7).

#### Bioreductive Anticancer Drugs

Hypoxic tumors have very poor prognosis and do not respond to existing chemotherapy thus requiring another therapeutic approach – bioreductive drug activation. The principle of bioreductive drug activation is that in the reductive environment created by hypoxia, endogenous reductases will transfer electrons to a nontoxic prodrug to generate a

reduced cytotoxic metabolite (28). Hence, this provides for differential killing of hypoxic tumors versus normal tissues. Here are some typical examples:

- mitomycin C (19).
- aromatic and heterocyclic nitro compounds (RSU1069, CB1954, pimonidazole) (29).
- aliphatic *N*-oxides: AQ4N (Novacea) is activated by CYP3A4 only under hypoxic conditions to form the active topoisomerase inhibitor AQ4 (30).
- benzotriazine di-*N*-oxides derivatives (tirapazamine) (31).
- Recent experiments showed that combining selective downregulation of cytochrome P450 reductase with newly synthesized tubulysin analog conjugated to 2-nitroimidazole (code name KEMTUB012-NI2) shows higher efficacy (1000 times more potent than tirapazamine) toward hypoxic tumor cells with limited systemic toxicity (31).

#### ***Gene Therapy: Targeting Drug and Enzyme to the Tumor***

Confers on tumor cells the genetic capacity to activate a pro-drug locally, within the tumor, and is designed to increase antitumor activity while minimizing toxic side effects to critical host tissues (32).

- Intratumoral injection of plasmid DNA, or retrovirus encoding P450.
- Localized delivery of P450-expressing cells encapsulated in cellulose sulphate.
- Use of human macrophages transduced with a hypoxia-regulated P450 cDNA.
- Intratumoral delivery of conditionally replicating oncolytic herpes virus encoding P450.
- Hypoxia-targeted gene therapy using an adenovirus that expresses P450R specifically under hypoxic conditions.
- Cyclophosphamide: a prodrug-activating P450 (CYP 2B6) gene is delivered to the tumor cell using a suitable gene therapy vector, providing for localized, intratumoral pro-drug activation and enhanced tumor cell killing (29).

#### ***Cytochrome P450 Enzymes as a Mechanism of Drug Resistance***

Increased expression of CYP1B1 in patients with ovarian cancers leads to the development of drug resistance to anticancer drugs which are inactivated metabolically by CYP1B1. Such drugs are docetaxel, doxorubicin, paclitaxel (in treatment of ovarian cancer (33)), mitoxantrone and tamoxifen.

#### ***Small Molecule Inhibitors of Cytochrome P450s***

The use of specific low-molecular-weight chemical inhibitors

of CYP1B1 to modulate the cytotoxic profile of some anticancer drugs is still under experimental stage. Examples: a methylated derivative of oxyresveratrol (piceatannol); tetramethyl stilbene; O-demethylation of biochanin A (a principle isoflavonoid found in red clover) by CYP1B1 produce genistein which in turns inhibited the enzyme. Another contemporary approach to enhance efficacy and reduce toxicity of anticancer drugs is their simultaneous delivery with some micellar cytochrome P450 inhibitors such as the well-known CYP3A4 inhibitor natural furanocoumarin 6',7'-dihydroxybergamottin (DHB). Intravenous application of DHB-micelles and docetaxel markedly increased antitumor efficacy in mice xenografted with MDA-MB-231 cells and showed enhanced plasma stability avoiding the reticuloendothelial system accumulation (34).

#### ***Antisense-Based P450 Therapy***

Further options for inhibiting the metabolism of anticancer drugs at their target site and in this way increasing their clinical efficacy are the antisense-based CYP strategies by suppressing or preventing CYP1B1 expression at the site of the tumor (1).

#### ***Immune-Based Therapy***

ZYC300 is a CYP1B1-based DNA vaccine, designed to stimulate the immune system against tumor cells expressing CYP1B1 (7).

#### ***Influence of Cytochrome P450 Polymorphisms on Drug Development and Anticancer Therapy***

Defining the profile of the individual tumors and identifying those CYPs that are overexpressed is of great importance to the successful future development of new anticancer drugs (35, 36). At the same time no less important for the clinician is to have clear cut information on the patient's cytochrome P450 phenotype, lowering in this way the inter-patient variability, minimizing side-effects and maximizing therapeutic efficacy (37-39). The advent of novel technologies such as tissue microarrays and protein chips should facilitate the potential of tailoring patient-specific therapeutic regimens based on individual CYP expression in tumor cells.

#### **CONFLICT OF INTEREST STATEMENT**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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