Pharmacogenomics of Cytochrome P450 Enzymes in Tumours

Diane Downie, Patrick H. Rooney, Morag C.E. McFadyen, Graeme I. Murray*

Department of Pathology, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK

Abstract: The field of pharmacogenomics is continuously progressing with the development and availability of molecular biotechnologies such as protein and DNA chips. These applications are pushing forward the frontiers towards individualised medicines and advanced predictions of an individual’s predisposition to diseases such as asthma, cardiovascular disease and cancer. The cytochrome P450s are an essential group of enzymes involved in metabolism of many currently administered drugs as well as foreign compounds and endogenous substrates. Inter-individual variation in response to cancer chemotherapy is often attributed to genetic alterations in this particular group of drug metabolising enzymes. Such alterations can impact on the pharmacokinetics and pharmacodynamics of anti-cancer drugs, especially those drugs with a narrow therapeutic index. The identification of variations is therefore, key in the areas of drug selection and dosage. Polymorphisms have been identified in many P450s including the CYP2C and CYP3A families, as well as CYP2D6 and CYP1B1. Polymorphisms in CYP2C and CYP3A family members have demonstrated altered cytotoxic drug potential while polymorphisms in CYP1B1 are considered potentially important in the etiology of a range of human cancers. However, the functional significance of P450 variants in tumours is largely unknown. Importantly, the identification of differential cytochrome P450 expression between tumour and corresponding normal tissue are key to the development of novel drugs and therapeutic strategies that can utilise the overexpression of these enzymes to their benefit. It is for these reasons that variants in the cytochrome P450 multigene family have become the focus of current research in this area.

Key Words: Pharmacogenomics, cytochrome P450, CYP1B1, polymorphism, anticancer agent, neoplasm.

INTRODUCTION

Pharmacogenomics is the study of how an individual’s genetic inheritance may affect the body’s response to drug treatments. The primary objective of pharmacogenomics is to provide drugs that have been tailor-made for individuals according to their own genetic make-up. With the reporting of the first draft of the human genome sequence [Lander et al., 2001; Venter et al., 2001], the future holds the potential for screening individuals from birth to establish their predisposition to diseases, including cancer. Ultimately, the sequencing of the human genome will provide a basis for understanding how variations in our basic genetic make-up can result in disease or a particular therapeutic outcome in later life. This approach will allow targeted monitoring of individuals with earlier intervention to prevent these diseases from occurring. Additionally, if a patient understands his predisposition to cancer, he is then in a more favourable position to make lifestyle changes, which may reduce his risk of developing cancer. A better understanding of an individual’s predisposition and genetic make-up will provide the clinician with tools to more accurately diagnose the patient’s symptoms and hence provide the key to more tailored therapy creating personalised drugs with greater efficacy and safety.

The advantages of such optimised drug therapy are considerable. Identification of drug resistance would no longer be a trial and error procedure eliminating potential adverse reactions. Dose optimisation would no longer be a lengthy process and these would ultimately lead to reduced healthcare costs. In 1994, a meta-analysis of 39 prospective studies from US hospitals revealed that more than 100,000 patients died from adverse responses to medications that were beneficial to others. Another 2.2 million experienced serious reactions, while others failed to respond at all [Lazarou et al., 1998]. DNA variants in genes involved in drug metabolism, particularly the cytochrome P450 multigene family, are the focus of much current research in this area. In this review the functional role that exists for genetic variability of cytochrome P450s, with regard to pharmacogenomics is outlined specifically considering implications of genetic variability of cytochrome P450s, with regard to the administration of anti-cancer drug treatment. The methods used to detect this genetic variability and its consequences on the design of novel therapeutic candidates, which operate within the tumour microenvironment, are discussed.

CYTOCHROME P450 FUNCTION, REGULATION AND EXPRESSION

The cytochrome P450 (CYP) enzymes are primarily endoplasmic reticulum-bound monooxygenases that metabolise many lipophilic, endogenous and xenobiotic substrates, including a large number of drugs and environmental chemicals [Estabrook, 1996]. Currently, the cytochrome P450 superfamily is comprised of 18 families with 57 members [Nelson, 2002]. A number of families are regulated by ligand activated transcription factors, in particular,
families 1-3 and to a lesser extent family 4. Enzymes encoded by these genes are responsible for much of the phase 1 metabolism of xenobiotics, including the majority of drugs in use today.

The CYP1 family, not only metabolise xenobiotics such as polycyclic aromatic hydrocarbons (PAHs), but also play an important role in specific bioactivation of anti-cancer drugs. CYP1 family substrates are capable of the upregulation of these enzymes through transcriptional activation. The presence of PAHs induces CYP1 activity and expression through activation of the aryl hydrocarbon receptor (AhR) [Whitlock, 1999]. Ligand binding between PAHs and the AhR results in the nuclear translocation of the AhR protein. Once inside the nucleus, AhR forms a heterodimer with the aryl hydrocarbon nuclear translocator (ARNT). The heterodimer complex then binds the xenobiotic response element (XRE) in the enhancer regions of CYP1A1 and CYP1B1, promoting the transcription of these genes [Ma, 2001; McFadyen et al., 2003]. Induction of CYP1B1 mRNA in human lymphocytes, in response to environmental carcinogens, has recently been proposed as a possible indicator of risk to lung cancer [Toide et al., 2003].

Although significant amounts of specific P450s are expressed in a range of normal extrahepatic tissues, the primary site of expression is the liver. Here the P450s play a central role in defense against harmful environmental chemicals [Henderson et al., 2000] in addition to determining the half-life and pharmacological properties of many therapeutic drugs. In the liver CYP3A4 is the most abundant P450 expressed, comprising approximately 30% of total human hepatic P450 [Plant and Gibson, 2003]. Of all prescribed drugs, 50-60% are thought to be metabolised by CYP3A4, which is regulated by the pregnane X receptor (PXR) [Willson and Kliwer, 2002].

In addition to normal tissue expression, specific P450s have also been detected in a range of tumour types including breast, liver, prostate and bladder [Murray, 2000; Patterson and Murray, 2002]. The identification of P450s, which demonstrate differential expression between normal and tumour tissues, provides potential targets around which determination of highly homologous rat cytochrome P450 enzymes CYP2B1 and CYP2B2 [Galeva et al., 2003]. Protein biochips offer the possibility of developing rapid global analysis of the entire proteome by analysing proteins in a format analogous to that of the DNA microarray. These arrays are currently used in drug discovery in applications including drug screening, profiling for diagnostics and target selection and new protein biochips are currently being developed for use in breast cancer research. It is anticipated that automation of the protein array will provide a basis for the analysis of clinical samples. In addition, they will be utilised in high throughput screening to supplement current available tools for the in vitro modelling of drug metabolism, such as organ slices, primary or immortalised cell lines, microsomal fractions, isolated or recombinant drug metabolising enzymes (DMEs) and DNA microarrays. Such biochips would comprise at least two assay components; one for DME expression profiling, and the other for DME activity measurements in the presence of test anti-cancer compounds.

PROTEIN BIOCHIPS: EMERGING TOOL FOR PROTEOMIC RESEARCH

An understanding of a patient’s complement of functionally active cytochrome P450 enzymes is critical for improved therapeutic intervention. Current approaches to identify these P450s are centered on techniques, such as mRNA-based expression profiling, antibody-based detection and the use of specific inhibitors or substrates. Current developments in technology such as matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry-based proteomics have provided a mechanism to identify distinct P450s. This technique allows identification of proteins by peptide-mass fingerprinting [reviewed by Aebersold and Mann, 2003]. The detection of multiple polymorphisms by this method implicates this as a cost-effective mechanism of high throughput phenotyping. This technology has recently been utilised to allow the differential identification of highly homologous rat cytochrome P450 enzymes CYP2B1 and CYP2B2 [Galeva et al., 2003].

Molecular profiling and advancing technologies role in molecular pathology

A plethora of methods, including laser capture microdissection, fluorescent activated cell sorting (FACS) and...
magnetic bead capture, have been developed to obtain cells of the same type, either from tissue or from blood. This allows researchers to perform analyses on homogeneous cell samples rather than the more heterogeneous mixed cell populations of larger tissues. This requirement to investigate disease processes at the single cell level, i.e. molecular profiling, provides a new and dynamic discipline, capable of generating a global view of mRNA, DNA alterations and protein patterns in various cell types and disease processes.

Currently, molecular-based drug discovery programmes are being employed to find new drugs. Molecular methods involve high throughput screening, often utilising microarray technology [Lennon, 2000]. Multiple applications exist for microarrays including comparative genomic analysis to identify chromosomal imbalances [Rooney et al., 1998], the study of mutations and genetic polymorphisms [Shi, 2001], and the study of gene expression [Macgregor, 2003]. Expression profiling is the most widely used application of microarray technology as the expression chip provides a mechanism to study the expression of thousands of genes simultaneously by comparison with a reference sample.

Microarray technology is currently being applied to better understand the molecular basis of many forms of cancer. The expression pattern of 7000 genes from breast cancer biopsies was analysed using oligo microarrays. Of the genes studied, 18 demonstrated altered expression [Dressman et al., 2001]. cDNA microarray analysis of gene expression has also been carried out on normal and malignant human prostate tissue [Chaib et al., 2001]. Of 588 genes studied by array analysis, 15 were found to be differentially expressed in malignant compared with normal tissue. One of the genes reported to change was CYP1B1. In another study, the Atlas (TM) human cDNA array was used to screen for alterations in the expression of 600 genes in normal human bronchial epithelial (NHBE) cells and several lung carcinoma cell lines [Hellmann et al., 2001]. Differential expression of 17 genes was observed, including the upregulation of CYP1B1 in all four carcinoma cell lines compared to NHBE cells. Identification of such genes by this technology may provide future predictive biomarkers as well as highlighting novel therapeutic targets thus illustrating the potential for DNA microarrays in the field of drug discovery and development.

FUNCTIONAL VARIANTS OF CYTOCHROME P450 ENZYMES: SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)

There are pronounced inter-individual and inter-ethnic variations in the capacity of individuals to metabolise drugs and other xenobiotics. Some of these variations are the result of alterations in the amount and specific activity of individual cytochrome P450s expressed by the individual. Genetic variation in these enzymes has the potential to produce patient populations that can be classified as “extensive metabolisers” or “poor metabolisers” of drugs. Amongst the numerous P450 subtypes which exist, CYPs 2D6, 3A4/5, 1A2, 2E1, 2C9 and 2C19 play a more crucial role in the genetically determined responses to a wide range of drugs [Mancinelli et al., 2000]. The principal P450 enzymes with polymorphisms implicated in drug metabolism are highlighted in “Fig. (1)”. Variations can be the result of one or a number of changes in the gene structure of an individual P450, for instance, changes in the promoter region can alter the amount of a particular P450 expressed. Single base changes in the coding region of a specific P450 gene may alter the activity of that P450. Single nucleotide polymorphisms (SNPs – variations in the DNA sequence occurring in at least 1% of the population) may result in alternative splicing and protein truncations resulting in the absence of a particular P450 expressed in the tissue of certain individuals. Recent studies demonstrate that polymorphisms in the CYP genes can lead to altered substrate specificity of these proteins [Ingelman-Sundberg, 2001]. Alterations to the functionality of these proteins may be of clinical relevance depending on the outcome to substrate metabolism.

CYP3A enzymes are involved in the metabolism of a number of anticancer agents, including, ifosfamide, cyclophosphamide [Chang et al., 1993], paclitaxel [Harris et al., 1994] and docetaxel [Royer et al., 1996]. Both CYP3A4 and CYP3A5 show wide inter-individual variation which may have an important influence both on drug response and disease susceptibility. Analysis of the 5' flanking region of CYP3A5 has identified two linked polymorphisms in the transcriptional regulatory elements, which are associated with increased expression and activity of the gene [Paulussen et al., 2000]. Individuals with increased expression of CYP3A5 may therefore, have differences in drug clearance rate and altered responses to therapeutic treatment. Polymorphisms in CYP3A4 were initially described in connection with variability in the oxidation of nifedipine [Kleinbloesem et al., 1984]. A total of 28 SNPs have recently been identified in the CYP3A4 gene demonstrating inter-ethnic variability amongst the 9 groups studied [Lamba et al., 2002].

In addition to SNPs in CYP3 family members, the CYP2D6 gene has been identified as containing in excess of 70 allelic variations [Marez et al., 1997]. Such variability in this gene has resulted in a range of phenotypes from “poor metabolisers” with no CYP2D6 enzyme activity to
extensive metabolisers” [Yu et al., 2002]. However, most anticancer drugs are not subject to CYP2D6 metabolism [Le Guellec et al., 1993].

The CYP2C family metabolises a wide range of therapeutic agents. There are four members of the subfamily, CYP2C8, CYP2C9, CYP2C19, and CYP2C18 and of these CYP2C8, CYP2C9, and CYP2C19 are of particular clinical importance. CYP2C8 is the principal enzyme involved in the metabolism of the cytotoxic drug paclitaxel [Rahman et al., 1994; Sonnichsen et al., 1995]. Several genetic polymorphisms in CYP2C8 have been identified, at least one of which, CYP2C8*3, is defective in the metabolism of paclitaxel in vitro [Dai et al., 2001; Soyama et al., 2001; Bahadur et al., 2002]. It is therefore, of clinical importance to evaluate the pharmacokinetics of paclitaxel in cancer patients with respect to CYP2C8 variant alleles. Polymorphisms in CYP2C9 and CYP2C19 have also been characterised [Goldstein and Morais, 1994; Sullivan-Klose et al., 1996]. Polymorphisms in the CYP2C19 gene occur in around 35% of Caucasians and 18-23% of oriental populations [Sullivan-Klose et al., 1996]. CYP2C9 polymorphisms are less common with a frequency of only 1:500 [Inaba, 1990]. All four CYP2C enzymes have the potential to activate cyclophosphamide and ifosfamide to manifest their chemotherapeutic activity [Chang et al., 1997] however, it has been demonstrated that various CYP2C polymorphic variants can culminate in either an increased or decreased catalytic efficiency towards these two anticancer agents [Chang et al., 1997]. Moreover, one such variant, CYP2C9 Gly417 to Asp mutation, was found to lower catalytic efficiency toward cyclophosphamide whilst increasing efficiency toward ifosfamide illustrating further the advantages of genomic screening prior to therapeutic treatment.

POLYMORPHISMS AND CYP1B1 ACTIVITY

Polymorphisms in CYP1B1 may lead to an increase in catalytic activity towards steroid hormones and procarcinogens when compared with wild-type CYP1B1 [Shimada et al., 1999; Hanna et al., 2000], suggesting that polymorphisms in this gene may contribute to inter-individual susceptibility to cancers. Several CYP1B1 polymorphisms have been identified. Four of these lead to the amino acid substitutions: Arg by Gly; Ala by Ser; Leu by Val and Asn by Ser at codons 48, 119, 432 and 453 [Bailey et al., 1998; Stoilov et al., 1998]. Specific allelic variants in the CYP1B1 gene have demonstrated 2.4-3.4 fold higher catalytic efficiency than the wild type CYP1B1 with respect to 4 hydroxylation of estradiol [Hanna et al., 2000]. Rapid cycle polymerase chain reaction and parallel analysis with resonance energy transfer probes has been utilised as a rapid single-step genotyping method for CYP1B1 codon 432 [Bruning et al., 1999]. The Val432 CYP1B1 variant has demonstrated substantially higher catalytic activity towards a series of procarcinogens as well as estradiol [Shimada et al., 1999].

CYP1B1 POLYMORPHISMS AND CANCER

This polymorphism is also associated with an increased risk of colorectal cancer [Fritsche et al., 1999] breast cancer [Bailey et al., 1998] and ovarian cancer [Goodman et al., 2001] as well as being a putative susceptibility factor in smoking-related head and neck squamous cell cancer (HNSCC) [Ko et al., 2001]. A study of breast cancer patients (n=186) revealed that women with the polymorphism in codon 432 (Val-Leu) had a 2.3 fold elevated risk of breast cancer compared with the case controls (n=200) [Zheng et al., 2000]. Additionally, this polymorphism at codon 432 was detected in 34% of prostate cancer subjects compared to 12% of matched controls [Tang et al., 2000].

The Ala-Ser polymorphism at codon 119, in the substrate recognition site 1, has also been associated with an increased incidence of breast cancer, squamous cell carcinoma of the lung [Watanabe et al., 2000] and prostate cancer [Tanaka et al., 2002]. Ethnic related differences in this and other CYP1B1 polymorphisms (Arg48Gly, Leu432Val and Asn453Ser) have been observed in Caucasian and Japanese populations, with a higher incidence of each in the Caucasian population [Inoue et al., 2000]. However, there are also studies which demonstrate no association between CYP1B1 polymorphisms and breast cancer risk [Rylander-Rudqvist et al., 2003] or lung cancer risk [Han et al., 2003] suggesting the overall effect of CYP1B1 polymorphisms on cancer risk to be low.

The overexpression of CYP1B1 in tumour tissue highlights this as an important area for future research. A recently developed molecular model for CYP1B1 has been created based on homology with mammalian CYP2C5 [Lewis et al., 2003]. It is anticipated that such a model will enhance knowledge of substrate structure activity relationships for CYP1B1 and its allelic variants, and would potentially identify future cytotoxics reliant on this P450 for activation within the tumour microenvironment.

A cytochrome P450 eSensor (TM) DNA Biochip Assay is now available to detect mutations in the Cytochrome P450 gene superfamily [Lenigk et al., 2002]. The assay is currently utilised in clinical trial studies of candidate therapeutics. The chip will be used initially to gather data to determine the importance of genetic mutations associated with a patient's ability to metabolise particular drugs. This information will assist pharmaceutical companies in correlating adverse reactions with a particular genotype during early phases of clinical trials, and so streamline selection of patients for the more costly later phases, to focus on those who will most benefit from a new drug.

NOVEL THERAPEUTIC OPTIONS AND OPPORTUNITIES ARISING FROM P450 OVER-EXPRESSION IN TUMOURS

As mentioned previously P450s are expressed in a range of normal tissues most of which are exposed to foreign toxins. However, the expression of specific P450s has also been detected in a range of tumour tissues including those of bladder, breast, colon, esophagus, kidney, lung, ovary, prostate, soft tissue sarcoma and stomach [Murray, 2000; Patterson and Murray, 2002]. This overexpression of P450 family members can and does influence the metabolism of anti-cancer drugs. P450s have been studied in a variety of human tumours using many different techniques. Recent
work utilising an osteosarcoma tissue microarray, with 18 primary tumours, demonstrated the expression of CYP1A1/2, 1B1 and 3A4/5, but not CYP2B6 and 2D6 [Dhaini et al., 2003]. Such screening and identification of P450s in this and other tissues would provide clinically relevant information, acting as predictive biomarkers to therapeutic response, as well as influencing future therapies.

Some anti-cancer drugs are administered as prodrugs, which require enzymatic transformation for activation. An ideal cancer chemotherapeutic prodrug is completely inactive until metabolised by a tumour-specific enzyme, or by an enzyme that is only metabolically competent towards the prodrug under physiological conditions unique to the tumour. Several strategies are based on the tumour expression of CYPs, including identification of prodrugs activated by tumour-specific polymorphic CYPs, use of CYP-gene directed enzyme prodrug therapy (GDEPT) and CYPs acting as reductases in hypoxic tumour regions [Patterson et al., 1999; Chua et al., 2000; Kan et al., 2002].

The following discussion demonstrates how the presence of P450s within tumour cells along with the knowledge of functional P450 variants in these cells can influence the efficacy of a chemotherapeutic agent, depending on the particular enzyme present.

**‘PHORTRESS’**

Phortress is one example of an anti-cancer drug that is administered as a prodrug. Phortress, is a derivative of the 2-(4-aminophenyl)benzothiazoles, a new class of chemotherapeutic agent with the ability to induce the P450 enzyme responsible for its bioactivation “Fig. (2)”. This new class of chemotherapeutic has demonstrated inhibitory activity against a panel of breast cancer cell lines in vitro [Shi et al., 1996] as well as ovarian, renal and colon cancer cell lines [Bradshaw et al., 1998]. CYP1A1 appears to be essential for the metabolism and antitumour activity of these chemotherapeutics [Chua et al., 2000].

2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (Phortress) has become the favoured analogue for clinical consideration from this new group of cytotoxic. Phortress has been conjugated to a lysine group to form a prodrug improving its overall bioavailability [Bradshaw et al., 2002]. Pro-Phortress is water soluble, chemically stable and undergoes rapid quantitative bioconversion to the parent moiety (Phortress) in vitro and in vivo [Bradshaw et al., 2002; Hutchinson et al., 2002]. Phortress is a potent agonist of the cytosolic AhR. The resulting AhR/ARNT heterodimer binds the XRE upstream of the CYP1A1 promoter [Loaiza-Perez et al., 2002]. It is this induction of CYP1A1 which is responsible for the bioactivation of Phortress within the tumour cells.

Phortress delivers its cytotoxic potential by causing DNA adduct production and apoptosis in sensitive tumours. As the cytotoxic potential of Phortress is dependent on induction of CYP1A1 via the AhR, this therapeutic strategy will be limited to tumours with a functional AhR signalling pathway. The induction, and metabolic properties of the closely related family member CYP1B1 in response to related compounds suggest a putative but undefined role for CYP1B1 in the mechanism of action of aminophenyl-

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![Fig. (2). Pro-Phortress is bioactivated to Phortress within the cell. Phortress is a potent agonist of cytosolic aryl hydrocarbon receptor (AhR). Following nuclear translocation, AhR forms a heterodimer with the aryl hydrocarbon nuclear translocator (ARNT). The resulting AhR/ARNT heterodimer binds the xenobiotic response element (XRE) upstream of the CYP1A1/1B1 promoter. The induction of CYP1A1 is responsible for the bioactivation of Phortress within the tumour cells. Induction of CYP1B1 and its ability to metabolise related compounds suggest a putative but undefined role for CYP1B1 in the mechanism of action of aminophenylbenzothiazoles.](Image)
benzothiazoles [Chua et al., 2000]. CYP1B1 could be a preferential activator of Phortress over CYP1A1 due to the over-expression of this enzyme in tumours [Murray et al., 1997; McFadyen et al., 2001a]. This could prevent the undesirable bioactivation of Phortress outwith the tumour environment. Phase 1 clinical trials are due to commence in 2003/2004.

AQ4N

Hypoxia in tumours is defined as the chronic or acute lack of oxygen within a tumour mass and is the result of the incomplete formation of the tumour vasculature. Hypoxic cells pose a problem to the control of solid tumours as they are less sensitive to a range of chemotherapeuticagents and are several times more resistant to radiotherapy [Brown, 2000]. AQ4N, an alkylaminoanthraquinone N-oxide, is a novel prodrug of a topoisomerase II inhibitor that is reductively metabolised in hypoxic cells to the active agent AQ4 [Patterson, 1993]. The reduction product, AQ4, demonstrates a 1000 fold increase in cytotoxic potency and remains stable under aerobic conditions. Additionally, the DNA affinic nature of AQ4 should ensure localisation within the tumour tissue.

The metabolic reduction of AQ4N is mediated by the NAD(P)H dependent cytochrome P450’s and other haemoproteins [Patterson, 1993]. The enzymology of AQ4N metabolism was first studied using a panel of human liver microsomes with known P450 phenotype and activities [Raleigh et al., 1998]. This work illustrated that anaerobic metabolism of AQ4N to the reduced tertiary amine AQ4 occurred in all 17 human liver samples, but was undetectable under aerobic conditions. The use of CYP inhibitors suggested CYP3A to be the major contributor in AQ4N reductive metabolism [Raleigh et al., 1998]. As CYP3A is detectable in a range of human cancers it is likely that this subfamily of enzymes is responsible for the selective activation of AQ4N in hypoxic tumours. Using a commercial source of recombinant cytochrome P450’s, CYP3A4 was found to metabolise AQ4N [Patterson et al., 1999]. CYP1A1, 1A2 and 2B6 were also found to play a role in AQ4N reduction [Robson and McKeown, unpublished observations].

When administered in combination, AQ4N can significantly enhance the anti-tumour effect of radiation [McKeown et al., 1996] and cyclophosphamide [Friery et al., 2000] as well as potentiating the activity of cisplatin and thio-TEPA [Patterson et al., 2000; Gallagher et al., 2001]. Although both normal and tumour cells are capable of AQ4N reduction, the process requires low oxygen [Patterson et al., 1999], therefore, this process should be restricted to hypoxic tumour cells. Phase I trials for AQ4N utilising a single dose, dose-escalation schedule are being carried out presently. AQ4N is the only CYP-activated bioreductive drug in clinical trials.

P450-BASED GENE DIRECTED ENZYME PRODRUG THERAPY (GDEPT)

A number of established prodrugs, such as cyclophosphamide and ifosphamide, undergo activation by cytochrome P450s located in the liver. Enhanced chemosensitivity of tumours can be achieved by gene transfer of P450s prior to administration of the prodrug thus enabling activation of the prodrug directly within the target tissue [Kan et al., 2002]. Various approaches have been utilised to deliver the P450 to the tumour site. This increase in cytotoxic activity increases the therapeutic effect of the administered drug without a corresponding increase in host toxicity. P450 GDEPT also creates a ‘bystander’ effect, extending the cytotoxic effect beyond those cells transduced with the prodrug activating P450 gene. P450 GDEPT prodrug substrates are dependent on various P450 genes. These substrates include investigational and well-established prodrugs as well as bioreductive prodrugs such as AQ4N, which, as mentioned, is activated primarily by CYP3A4 in a hypoxic tumour environment [McCarthy et al., 2003].

FREE RADICALS IN CANCER THERAPY

Free radicals are produced naturally within the body. They are usually highly reactive and short-lived, and have the capacity to damage DNA. Their ability to cause DNA damage has led to interest in utilising these free radicals in cancer therapy. These therapies are being designed around prodrug methodology with the ability to deliver damaging radicals to tumours. As with many current prodrug developments this would rely on the enzyme/prodrug interaction although in this scenario the end result would be the production of toxic radicals as opposed to cytotoxic drugs [Wardman CRC Scientific Yearbook 2001-02]. The targeting of these prodrugs towards enzymes that are specifically expressed in tumour tissue, such as CYP1B1, would, potentially, offer an enhanced therapy [Murray et al., 1997].

PRODRUG ACTIVATION

The ability to tailor cytotoxic agents, which will act at the tumour site, remains vital in the treatment of cancer. The cytochrome P450 enzymes capable of activating prodrugs such as the alkylating agents cyclophosphamide and ifosphamide, provide an excellent target for selective kill at the site of the tumour. To date, the majority of studies have been restricted to human cell lines and animal models. The approach generally consists of tumour-specific conversion of prodrugs to active drugs following delivery of genes for exogenous enzymes. For example, intra-tumoural expression of CYP2B1 sensitises tumour cells to the cytotoxic action of agents such as cyclophosphamide and ifosphamide which are generally metabolised in the liver to the 4-hydroxylated metabolites [Chen and Waxman, 1995]. These metabolites undergo spontaneous decay to phosphoramide mustard and acrolein which alkylate protein and DNA respectively. The short half-life of these metabolites makes this an ideal approach for targeting drugs to tumour cells and avoiding any possible diffusion of toxic products to normal non-tumour tissue.

The results of a phase I/II trial in which 14 patients with inoperable pancreatic cancer had microencapsulated genetically modified allogenic cells containing CYP2B1 delivered to the tumour vasculature followed by ifosphamide...
administration have recently been published [Lohr et al., 2001]. The results of this small study compared favourably with a previous trial to assess the effectiveness of the cytotoxic agent gemcitabine in patients with advanced pancreas cancer [Burris et al., 1997]. The group of patients receiving the microparticle encapsulated cells had a mean survival double that of the control group with a 1 year survival three times that of the control group. The finding that individual cytochrome P450s may be differentially expressed in tumour tissue compared with normal tissue highlights these enzymes as potential targets for enzyme-directed drug therapy. In particular, CYP1B1 may represent a tumour marker to which therapeutic compounds may be directed. This P450 is over-expressed in tumour cells but is barely detectable in normal tissue even though CYP1B1 mRNA is present [Edwards et al., 1998; McFadyen et al., 1999].

CYP1B1 AS A UBQUITOUS TUMOUR MARKER AND DRUG TARGET

There are several possible therapeutic avenues that may be used to exploit the tumour-specific expression of CYP1B1. These include the development of a prodrug specifically activated by CYP1B1 metabolism, the development of low-molecular weight inhibitors of CYP1B1 (designed to stop catalysis of co-administered cytotoxic drugs) or use of the CYP1B1 tumour antigen for diagnostics and the development of immunotherapeutic strategies.

CYP1B1-ACTIVATED PRODRUGS

The development of prodrugs, which are inactive until converted to the cytotoxic form by CYP1B1, would be superior to the prodrug approaches in use at present. Currently, prodrugs such as cyclophosphamide are activated in the liver by CYP2B6 and CYP3A4 [Chang et al., 1993; Bohnenstengel et al., 1996]. However, a CYP1B1 prodruag-activated approach would ensure that the prodrugs remain inactive until inside the cancerous cell, whereupon they would be activated to a cytotoxic form.

CLINICAL INHIBITORS TO CYP1B1

Studies in vitro have highlighted the involvement of CYP1B1 in tumour specific anticancer drug inactivation [McFadyen et al., 2001b; Rochat et al., 2001]. A recent study confirmed the finding that CYP1B1 interacts with docetaxel and results in a reduced cytotoxic potential [Bournique and Lemarie, 2002]. Specific inhibitors to CYP1B1 have been used previously to modulate the cytotoxic profile of a range of structurally diverse anticancer drugs with CYP1B1 [McFadyen et al., 2001b, Rochat et al., 2001]. The stilbene trans-resveratrol has recently been found to inhibit the catalytic activity of CYP1B1 as well as suppressing its constitutive gene expression [Chang et al., 2000]. However, resveratrol also acts as a competitive inhibitor of CYP1A1 [Chun et al., 1999]. Tetramethoxy-stilbene (TMS), a resveratrol analogue, is the most selective and potent CYP1B1 inhibitor to date [Chun et al., 2001]. Unlike resveratrol, TMS exhibited 50-fold and 500-fold selectivity for CYP1B1 over CYP1A1 and CYP1A2 respectively [Chun et al., 2001]. In cultured human colon cells TMS potentiated the inhibition of cancer cell growth implicating a future role for this inhibitor as a cancer chemotherapeutic or cancer chemopreventive agent [Nam et al., 2001]. Despite the benefits of clinical inhibitors further studies in this field are required to establish whether CYP1B1 allelic variants posses the same response to these inhibitors as wild type CYP1B1.

Clinical studies with ovarian cancer patients have revealed that patients with a weak tumour expression of CYP1B1 were found to have increased survival, in response to docetaxel treatment, when compared to those patients expressing greater amounts of the enzyme [McFadyen et al., 2001a]. Such studies potentially provide the rationale for the development of small molecule inhibitors to CYP1B1. Used in combination with anti-cancer drugs, inhibitors may increase the efficacy of cancer treatment “Fig. (3)”.

CYP1B1-DIRECTED IMMUNOTHERAPY: NOVEL ANTI-CANCER TREATMENT ZYC300

The specific localisation of CYP1B1 to tumour cells and its ability to interact with anti-cancer drugs is now being targeted by immune-based therapy, a technology dependent upon stimulating the cancer patient’s immune system against their tumour. ZYC300 (Zycos Inc, Lexington Massachusetts) is a novel anti-cancer treatment currently undergoing phase 1/2a trials at the Dana Faber Cancer Institute. Clinical trials are being carried out on a number of patients with breast, ovarian, colorectal and prostate cancer. ZYC300, a CYP1B1 DNA vaccine, has been designed by ZYCOs to stimulate the body’s immune response against cells expressing CYP1B1. CYP1B1, although structurally similar to other P450’s, shares only approximately 40% homology to the closest P450 family member. This, along with the selective expression of CYP1B1 protein in tumour cells [Murray et al., 1997], makes CYP1B1 a unique molecular target for anti-tumour therapeutic development. Preliminary data has indicated that ZYC300 is capable of inducing a specific T cell response to destroy cancer cells. ZYC300 employs the BIOTOPE® expression cassette system, whereby plasmid DNA, which encodes the candidate antigen sequence, is encapsulated within a biocompatible polymer microparticle. It has been shown that a partial DNA sequence introduced in this manner allows for better delivery to specialised antigen presenting cells (APCs) such as the dendritic cells and macrophages, which phagocytose the microparticles [Hedley et al., 1998]. Effective delivery to the APCs can enhance both B cell and cytotoxic T cell response, a feature lacking in direct DNA injection approaches [Pardoll and Beckerleg, 1995]. The presentation of CYP1B1 peptide by receptors on the surface of APCs triggers the activation of immune cells which should ultimately result in the overall destruction of CYP1B1-expressing tumour cells.

The ability to target CYP1B1 with prodrugs, clinical inhibitors and immunotherapy highlights the importance of this unique molecule as an important cancer target. The fundamental biological question which still requires an answer is: what are the mechanisms switching on and maintaining CYP1B1 expression in histologically diverse tumours? It is important to be aware that while the above
discussion relates to the targeting of CYP1B1 other, as yet unidentified, P450s may exist which like CYP1B1, may be overexpressed in tumour. As a result these therapeutic strategies may become applicable to other candidate P450s as our understanding of the P450 multigene family increases.

**DISCUSSION**

Pharmacogenomics will ultimately lead to a shift from the current strategy of developing drugs optimised for a fraction of patients to the provision of tailored medications for genetically diverse patients. Pharmacogenomics has received new impetus from the development and accessibility of molecular biotechnologies. The ability to predict phenotypic outcomes from gene expression profiles is currently in its infancy, however, evolving oligonucleotide microarray technologies and genome sequencing will provide quantitative assessment of expression profiles of genes in a range of tissues. The development of gene expression profiling and the understanding of the regulation and the signal transduction mechanisms elicited by pharmaceutical agents will be a major step towards predictive, individualised medicine. However, to achieve a clinically useful and predictive level in pharmacogenomics, much work remains.

The diagnostic and therapeutic implications of P450 in tumours are becoming increasingly apparent. The identification of differentially expressed P450s by molecular profiling may provide future predictive biomarkers as well as defining targets for future therapies. Additionally, identification of tumour P450s is important due to their potential to metabolise anti-cancer drugs. This metabolism generally results in detoxification, although in a few cases the result is bioactivation. Early identification of P450s in a particular tumour will provide a key to the therapies best suited to treat the cancer (either a drug which is not detoxified by the enzymes present or identification of a tumour specific P450 which may be targeted to activate a specific drug). An improved knowledge of substrate structure activity relationships for the P450 multigene family is a necessity for the development of future anti-cancer drugs. Unfortunately, as is the case with existing cancer therapies, the evolutionary nature of the cancer cell is such that resistance mechanisms will inevitably be encountered adding to the difficulty of targeted therapies such as those mentioned in this review.

To date, several cytochrome P450 enzymes have been shown to be polymorphic as a result of single nucleotide polymorphisms, gene deletions and gene duplications. It is important to identify genetic variation in normal and tumour tissue and establish the functional significance of the P450 variants in tumours as genetic variability in P450 enzyme activity can lead to inter-individual differences in response to treatment. In many cases, these polymorphisms impact on the pharmacokinetics, metabolism, safety and efficacy of drugs. Therefore, genetic variations are now playing an increasing role in drug discovery, particularly in disease-specific drug target identification and in drug candidate profiling to predict drug response in genetically heterogeneous patient populations. Awareness and application of this knowledge will improve drug use in clinical practice and provide the clinician with further appreciation that standard drug dosing may not be appropriate in all patients. Although in its infancy, studies of
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P450 polymorphisms are now revealing that certain polymorphic variants may be correlated with disease etiology and drug response. As this area advances, early screening for such variants may become increasingly important to enhance awareness to an individual’s predisposition to cancer and outcome in response to treatment. Together, advances in the profiling of P450s and their respective variants may revolutionise current chemotherapeutic strategies as highlighted by the many novel therapeutic options arising from P450 over-expression in tumours.

REFERENCES


