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Differential Regulation of CYP3A4 and CYP3A5 and Its Implication in Drug Discovery

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Abstract

Cancer cells use several mechanisms to resist the cytotoxic effects of drugs, resulting in tumor progression and invasion. One such mechanism capitalizes on the body's natural defense against xenobiotics by increasing the rate of xenobiotic efflux and metabolic inactivation. Xenobiotic metabolism typically involves conversion of parent molecules to more soluble and easily excreted derivatives in reactions catalyzed by Phase I and Phase II drug metabolizing enzymes. Recent reports indicate that components of the xenobiotic response system are upregulated in some diseases, including many cancers. Such components include the pregnane X receptor (PXR) and the cytochrome P450 (CYP) 3A4 and 3A5 enzymes. The CYP3A enzymes are a subset of the numerous enzymes that are transcriptionally activated following the interaction of PXR and many ligands. Intense research is ongoing to understand the functional ramifications of aberrant expression of these components in diseased states with the goal of designing novel drugs that can selectively target them.

Keywords

Cancer; cytochrome P450; CYP3A4; CYP3A5; drug–drug interactions; drug resistance; drug discovery

1. INTRODUCTION

Humans have evolved robust mechanisms that simultaneously modulate the efficacy and toxicity of drugs. Such mechanisms can mitigate the disastrous consequences associated with exposure to harmful xenobiotics, such as environmental pollutants. Central to the xenobiotic response system are the cytochrome P450 (CYP) hemoenzymes, which also play

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CONFLICT OF INTEREST

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prominent roles in the metabolism of endogenously produced molecules (i.e. endobiotics). Although the liver is the major site of CYP biosynthesis in healthy adults, variable levels are expressed in extrahepatic tissues and organs, including the small intestine, kidney, lung, and brain [1–6]. Many cancers are characterized by an altered expression pattern of CYPs, in which expression may be either elevated or suppressed in different tumor types [7–13].

CYPs are predominantly monooxygenases that mediate hydroxylation and oxidation reactions that promote hydrophilicity of CYP substrates, resulting in either activation of prodrugs or enhanced clearance and excretion of chemotherapeutic agents by the kidneys [14]. The catalytic activity of CYPs markedly contributes to rapid cancer-drug clearance and concomitantly reduces systemic exposure and efficacy, as well as the possible generation of toxic drug metabolites. Therefore, methodical evaluation of these biological outcomes is an integral aspect of CYP-targeted drug discovery.

In humans, approximately 60 CYP proteins have been identified, which are classified into 18 families [15–17]. Current evidence suggests that the CYP1, CYP2, and CYP3 families collectively mediate the biotransformation of most xenobiotics, whereas CYPs in other families are involved in endobiotic metabolism [18–23]. Indeed, approximately 80% of all clinical drugs are primarily metabolized by members of the CYP1, CYP2, or CYP3 families, and 50% are exclusively metabolized by members of the CYP3A subfamily [23].

The human locus for the CYP3A gene cluster is located on chromosome 7q21-q22. It comprises two pseudogenes (*CYP3AP1* and *CYP3AP2*) and four genes (*CYP3A4*, *CYP3A5*, *CYP3A7*, and *CYP3A43*). The arrangement of CYP3A gene cluster is depicted in Figure 1. Each of the four functional genes at the CYP3A locus comprises 13 exons. The *CYP3A4* gene is located upstream from *CYP3A43*, which is furthest from the centromere and is the only gene in the cluster that is in the opposite orientation [24–28]. A pseudogene (*CYP3AP2*) separates *CYP3A4* from *CYP3A7*, and another (*CYP3AP1*) separates *CYP3A7* from *CYP3A5*. The *CYP3A5* gene is first in the cluster and closest to the centromere (Figure 1). The vast number of SNPs that affect each gene, especially *CYP3A4* and *CYP3A5*, are not restricted to any specific portion but are found in all parts of the gene structure, including 5' upstream regions, introns, exons, and the 3' untranslated regions (UTRs) [29,30].

The four functional genes at the CYP3A cluster encode sequence-related proteins that markedly differ in tissue distribution and expression level [24,26,27]. CYP3A43 is the major subfamily protein expressed in the testis and prostate, whereas CYP3A7 is mainly expressed in fetal liver, placenta, and endometrium [25,31–35]. CYP3A4 is the most studied isoform in the CYP3A subfamily and is predominant in the small intestine and adult liver. It is responsible for the bulk of metabolic activities ascribed to the CYP3A subfamily. Although less is known about CYP3A5, it is the most abundant isoform expressed in the kidney and lungs [36–42]. CYP3A5 is also polymorphically expressed in adult liver, but the expression level is highly dependent on the individual's genotype as will be discussed in more details in Section 2.2.1.

Many CYPs are induced by various xenobiotics, including therapeutic agents. Recent reports strongly suggest that management of certain cancers is compromised by drug-induced expression of members of the CYP3A subfamily [43]. CYP3A5, which is not highly expressed in disease-free pancreas, is abundantly expressed in exocrine-like pancreatic ductal adenocarcinoma (PDAC) but not in quasi-mesenchymal and classical PDAC subtypes [9]. Noll and colleagues reported a dramatic increase in CYP3A5 levels following treatment of exocrine-like PDAC with tyrosine kinase inhibitors. Therefore, they concluded that the resistance of exocrine-like PDAC is associated with the drug inducibility of CYP3A5.

It cannot be definitively concluded that CYP3A5 plays a causal role in neoplastic drug resistance considering that its expression pattern in unaffected tissues remains relatively unstudied. Many studies are limited by the assumption that surrounding nontumor tissues are adequate controls for comparing the expression or downregulation of proteins in nearby tumors. In addition, both CYP3A4 and CYP3A5 share approximately 80% structural similarity and have largely overlapping functions, making the isoforms difficult to distinguish. Moreover, activation of the pregnane X receptor (PXR; NR1I2), which is known to alter the expression profiles of both CYP3A4 and CYP3A5 in the liver, affects neither the basal expression nor inducibility of CYP3A4 in the pancreas [9]. This finding challenges the hypothesis that both isoforms share the same regulatory pathways [44,45]. Therefore, in this review, we will summarize the evidence distinguishing CYP3A4 and CYP3A5 function and activity. We will also examine the probable mechanisms of differential regulation and the rationale for their consideration as therapeutic targets in drug discovery.

2. EXPRESSION AND ENZYMATIC ACTIVITIES OF CYP3A4 AND CYP3A5

2.1. Expression profile of CYP3A4 and CYP3A5 in cancerous and noncancerous tissues

The expression levels of CYP3A4 and CYP3A5 are higher in many cancers than in comparable nontumor tissues (Table 1). This raises the question of whether both isoforms have functions that affect the development, proliferation, and metastasis of malignant tumors, making CYP3A4 and CYP3A5 promising anticancer drug targets. Indeed, elevated CYP3A4 levels in some breast cancers may promote tumor progression, resulting in poor survival outcomes of patients [46,47]. Mitra *et al.* suggested that CYP3A4 possesses active epoxigenase activity that converts arachidonic acid to epoxyeicosatrienoic acids, which promote breast cancer cell growth in a Stat3-dependent manner [46]. Similarly, high expression levels of CYP3A5 are present in estrogen receptor-positive breast cancers, but it is not known if the epoxigenase mechanism described for CYP3A4 is involved in the enhanced proliferation of these cancer cells [48].

In the prostate, CYP3A5 conventionally catalyzes the 6 β -hydroxylation of testosterone to its lesser active derivative, 6 β -hydroxytestosterone [49]. Several reports indicate that healthy prostate tissues express high basal levels of CYP3A5, but the evidence for altered CYP3A5 levels in cancerous prostate tissues is inconclusive [50–52]. Although a recent study suggested that CYP3A5 contributes to the nuclear accumulation of the androgen receptor, driving the progression of prostate cancer, the study did not define the exact mechanism of CYP3A5-mediated androgen receptor translocation [49,53].

CYP3A5 may be downregulated in some cancers, such as hepatocellular carcinomas (HCCs) [54–57]. The most recent findings suggest that CYP3A5 impedes HCC metastasis by inhibiting the ROS/mTORC/AKT signaling pathway, which regulates cell growth, survival, and migration. Therefore, reduced CYP3A5 expression may serve as an adaptation mechanism for HCC cell survival.

2.2. Molecular mechanisms that underlie aberrant expression of CYP3A4 and CYP3A5

2.2.1. Single nucleotide polymorphisms and copy number variations—A major factor affecting the expression and activity of CYP3A4 and CYP3A5 is the presence of single nucleotide polymorphisms (SNPs). SNPs are genetic conditions in which a position in a gene is occupied by different nucleotides, and each resultant variation is found within the population. A comprehensive presentation of the nomenclature and annotation of the CYPs and the various SNPs that affect them are available at www.cypalleles.ki.se/ and the Single Nucleotide Polymorphism database (dbSNP). The biological consequences of some SNPs can result in stark variations in enzyme expression and activity, which, in turn, may drive the disparities in interindividual drug response, toxicity, bioavailability, and clearance.

The wild-type allele of *CYP3A4* (i.e. *CYP3A4*1A*) is predominant in the general population, whereas the first identified variant, designated in the dbSNP as rs2740574 (i.e. *CYP3A4*1B*, which has an A>G substitution in the 5' promoter region) is present in 66%, 4%, and 0% of African-American, European, and Asian populations, respectively [58–62]. The finding that *CYP3A4*1B* is present in men with a more severe clinical stage and grade of prostate cancer led some investigators to speculate that clinical prognosis is associated with the presence of this allele [63]. However, experimental data indicate that SNPs in the promoter region of *CYP3A4* do not greatly impact its expression level or activity [61,64]. Although approximately 20 SNPs occur within the coding region of *CYP3A4*, little is known about their effects on expression and activity of the enzyme. However, a variant with a base insertion resulting in a premature stop codon, rs67666821 (i.e. *CYP3A4*20*) produces a truncated protein that does not incorporate heme and is, therefore, nonfunctional [65].

Similar to *CYP3A4*, *CYP3A5* has several allelic variants, with variable expression levels and activities. *CYP3A5*1* is regarded as the wild-type allele, and several reports indicate that individuals who have at least one copy express it at greater levels than do those who are homozygous for other variants that affect the coding region [30,61]. At the other extreme is *CYP3A5*3*, which is designated as rs776746 in the dbSNP. It is produced by alternative splicing caused by a 6986A>G SNP in intron 3. This creates a novel exon and premature stop codon, which results in expression of a truncated protein [66–68]. Although the novel splice site in *CYP3A5*3* is preferentially used in homozygous individuals, attenuated levels of correctly spliced CYP3A5 mRNA and functional protein are still produced [45,51,61]. See Lakhman and colleagues for exhaustive, yet elegant, reviews of other SNPs that affect the expression and activity of CYP3A4 and CYP3A5 [30,69].

Susceptibility to some cancers may be affected by *CYP3A5* SNPs. Shimada *et al.* found that Japanese women who carry the *CYP3A5*1* allele have a higher risk of breast cancer than do women who are homozygous for *CYP3A5*3*. The investigators hypothesized that greater

levels of functional CYP3A5 protein augment the 16 α -hydroxylation of estrogen, which is a critical step in breast cancer development. In contrast, a homozygous *CYP3A5**3 genotype is hypothesized to be nonfunctional and, therefore, does not increase breast cancer risk [70]. Differences exist in the allele frequency of CYP3A5 variants, which may partly account for the different expression levels of CYP3A5 in different populations [40]. One study reports that 93% Canadians have the *CYP3A5**3 allele compared with 77% Zimbabweans [69].

Unlike some members of the CYP2 family, little is known about duplication or deletion of the *CYP3A4* and *CYP3A5* genes [71,72]. One study reported a 10-fold increase in the copy number of *CYP3A4* in one of 143 individuals from eight ethnically diverse populations [73]. This suggests that copy number variations do not contribute much to the complexity of the polymorphic variations in *CYP3A4* and, perhaps, *CYP3A5*.

2.2.2. Epigenetic factors—The two major types of epigenetic mechanisms that alter gene expression are DNA methylation and histone modifications [81]. One of the first indications that epigenetics contributes to the differential expression of CYP3A4 and CYP3A5 was observed in immortal HepG2 cells, which express high levels of CYP3A4 in response to the demethylating agent 5-aza-2-deoxycytidine [82]. DNA methylation within the promoter region of a gene generally suppresses its expression, and histone modifications affect chromatin structure, accessibility, and transcriptional activity of genes [83]. Recently, a study of CYP3A5 activity, expression, and polymorphisms in prenatal human liver found that extensive variability occurs among individuals, even after exhaustive genotypic classification [84]. The authors hypothesized that epigenetic alterations contribute to the interindividual variability of CYP3A5.

Although it has been suggested that DNA methylation is heritable and stable, some studies have shown that methylation changes occur throughout the lifetime of an individual and, thus, contribute to the phenotypic variation among individuals [85–88]. Indeed, colorectal cancers are characterized by decreased methylation of the PXR promoter, which results in increased PXR-dependent expression of CYP3A4 [89,90]. In addition, a study of the methylation state of the 12kb upstream promoter region of *CYP3A4* in the livers of 79 individuals revealed that the degree of methylation of some transcription-factor binding sites, such as those for hepatocyte nuclear factor 4 alpha (HNF4 α), were highly variable among the study cohort [28]. This could potentially lead to differential expression of CYP3A4 in the general population. More research is needed to determine the extent to which epigenetics contributes to the phenotypic variations in the expression and activity of CYP3A4 and CYP3A5 within a population.

2.3. Transcriptional and posttranscriptional regulation of CYP3A4 and CYP3A5

2.3.1. Transcription regulation of CYP3A4 and CYP3A5

2.3.1.1. Pregnane X receptor (PXR; NR1I2): PXR is an orphan nuclear receptor (NR), mainly synthesized in the liver and intestine, and has a highly flexible and hydrophobic ligand-binding domain, which enables it to bind a vast array of structurally diverse molecules [91–94]. PXR is activated by agonist binding. It interacts with retinoic acid receptor (RXR; NR2B1) to form a heterodimer that binds to a PXR response element in the

5' regulatory regions of several genes, including *CYP3A4* and *CYP3A5*, to induce their expression [95–97]. Transactivation is accentuated by the cooperative binding of enhancer molecules, particularly the PXR/RXR heterodimer, at the distal xenobiotic responsive enhancer module (XREM), which is upstream of the proximal PXR response element [98]. In addition, indirect activation of PXR can occur through inhibition of inhibitory posttranslational modifications, such as phosphorylation [99–101].

PXR expression is elevated in many human cancers compared with that in corresponding healthy tissues [102–109]. In certain cancers, such as osteosarcoma, breast, and colorectal cancers, there is a correlation between the expression levels of PXR and *CYP3A4* and/or *CYP3A5* [103,108,110]. In addition, recent findings suggest that PXR regulates drug-induced *CYP3A5* upregulation in some cancers, but the baseline expression is PXR-independent [9].

2.3.1.2. Constitutive androstane receptor (CAR; NR1I3): The constitutive androstane receptor (CAR) is another orphan NR that greatly influences the transcription of several *CYP* genes. Although CAR shares substantial structural and functional similarities with PXR, it binds far less chemical entities than PXR, partly because of its smaller ligand-binding pocket [94,111–114]. It exerts transcriptional control on its target genes in a similar way to PXR. Upon CAR activation, it forms a heterodimer with RXR. The heterodimer complex recognizes identical sequence motifs in the distal XREM and proximal promoter regions of its target genes, which include *CYP3A4* and *CYP3A5* [115,116]. The mRNA of CAR is highly expressed in human liver and kidneys but is weakly expressed or not expressed at all in various other tissues [115,117]. Recent reports also indicate that CAR is expressed at varying levels in some non-small cell lung cancer and brain cancer stem cells, but the mechanism of action does not appear to involve members of the *CYP* family [118,119].

2.3.1.3. Hepatocyte nuclear factor 4 alpha (HNF4α; NR2A1): The liver expresses high levels of the NR HNF4α, which can potentiate the transcriptional activities of other transcription factors [120]. HNF4α expression level varies in extrahepatic tissues, including the intestine, kidney, and pancreas [9,121,122]. HNF4α also plays a critical role in the transcriptional regulation of a number of enzymes involved in xenobiotic metabolism [123,124]. Expression of antisense HNF4 RNA in human hepatocytes results in reduced *CYP3A4* and *CYP3A5* expression [125]. Indeed, HNF4α augments the basal and PXR- and CAR-mediated upregulation of *CYP3A4* in liver-derived cells by binding to a specific response element in the distal XREM, which is upstream of the PXR- and CAR-response elements in the proximal promoter region of *CYP3A4* [126]. Similarly, basal expression of *CYP3A5* in exocrine-like PDAC is regulated by HNF4α [9].

2.3.1.4. Vitamin D receptor (VDR; NR1I1): The vitamin D receptor (VDR), PXR, and CAR are members of the NR1I subfamily. Unlike PXR and CAR, VDR is an NR that specifically binds its secosteroid ligand 1α, 25-dihydroxyvitamin D3, which is the hormonally active form of vitamin D3. Like PXR and CAR, the ligand-bound VDR forms a heterodimer with RXR, which then binds and activates the VDR response elements of its target genes, such as *CYP3A4* and *CYP3A5* [127,128]. VDR-dependent upregulation of

these two genes has been observed in intestinal and prostate cancer cells and in hepatocytes of patients with metastatic cancers (primary kidney and colon tumors) [129–133].

2.3.1.5 Glucocorticoid Receptor (GR; NR3C1): GR is ubiquitous in vertebrate cells and regulates a diverse array of cellular processes such as steroidal metabolism, stress response and inflammation in different cell types [134]. CYP3A4 expression in the lungs is neither induced by glucocorticoids nor any of its known inducers such as rifampicin, nifedipine and clotrimazole [41]. However, CYP3A5 is inducible in the lungs and some lung cancer cell lines by such glucocorticoids as dexamethasone, budesonide and beclomethasone dipropionate. [41,135]. Glucocorticoid-induced expression of CYP3A5 in the lungs is mediated by GR-dependent mechanisms. This is contrary to the PXR- and CAR-dependent mechanisms of glucocorticoid-induced expression of CYP3A4 in human hepatocytes [136].

2.3.1.6. Yin Yang 1 (YY1): Yin Yang 1 (YY1) is a ubiquitous zinc-finger protein that regulates gene expression, primarily through binding a preferred 5'-CGCCATNTT-3' sequence in the 5'-flanking regions of its target genes [137–139]. A YY1-binding site, which overlaps the PXR-binding site, is absent in the promoter region of *CYP3A5* but is present in that of *CYP3A4*. Binding of YY1 to this site on *CYP3A4* represses its transcription [140]. Nem *et al.* hypothesized that the lack of YY1 binding to the *CYP3A5* promoter is the basis for the differential expression between CYP3A4 and CYP3A5 in the kidneys, in which CYP3A4 expression is low or negligible. YY1 most likely binds to its cognate recognition motif on *CYP3A4*, thereby suppressing its expression in the kidneys. Nem and coworkers argued that the differential renal expression of CYP3A4 and CYP3A5 may be further accentuated by the absence of PXR in human kidneys, which offsets the inhibitory effect of YY1 by acting on response elements, such as XREM [92,117,141]. YY1 inhibition of *CYP3A4* in renal cells is not affected by binding of ectopically expressed PXR to the proximal promoter alone. However, inclusion of the XREM region of the *CYP3A4* promoter reverses YY1 inhibition. Thus ectopically expressed PXR promotes expression of both CYP3A4 and CYP3A5 in renal cells to the levels present in the intestine and other organs where PXR and both CYP isoforms are expressed. It is plausible to speculate that additional factors, such as HNF4α, might be involved in the expression of both isoforms in these tissues considering that YY1 inhibition is reversed when the XREM region, which is recognized by HNF4α, is included.

2.3.2. Posttranscriptional regulation of CYP3A4 and CYP3A5—Micro-RNAs (miRNAs) are ~22-nucleotide RNA molecules that base pair with complementary sequences in the 3' UTR of target genes to reduce protein expression through either translational repression or initiation of mRNA degradation [142,143]. Of the 114 miRNAs that could potentially alter the expression levels of CYP3A4, approximately 10% mediate decreased CYP3A4 expression in healthy and cancerous tissues [144–146]. The miRNA 27b (miR-27b) directly binds to the 3' UTR of *CYP3A4* and causes downregulation of the protein in pancreatic and colon cancer cell lines [144]. In addition, miR-24 and miR-34a indirectly affect the expression level of CYP3A4 by regulating the expression of HNF4α in HepG2 cells [145]. Furthermore, miR-148a-dependent inhibition of PXR indirectly regulates CYP3A4 levels in cancer cell lines as well as human liver samples [147] Research into the

regulatory potential of miRNAs on CYP3A4 and CYP3A5 expression is incipient, and more work is necessary, including using animal models and healthy and cancerous tissues, before conclusions regarding miRNA contribution can be made.

2.4. Regulation of enzymatic activity of CYP3A4 and CYP3A5

2.4.1. Structure and function of CYP3A4 and CYP3A5—A comparison of the primary sequence of members of the CYP3A subfamily shows that they are more than 70% identical, and the homology is as high as 84% between CYP3A4 and CYP3A5 (Figure 2). Although both CYP3A4 and CYP3A5 have broad specificities for a wide range of overlapping substrates, available crystal structures of free and complexed CYP3A4 provide a strong rationale for its much higher substrate promiscuity (Figure 3). The CYP3A4 active site can bind a large number of different substrates in multiple conformational states, as well as accommodate simultaneous binding of ligands [148–150]. The crystal structure of unbound or ligand-bound CYP3A5 is yet to be solved. Nonetheless, available information from comparative analysis, using molecular docking and simulation, suggests that there may be some subtle and distinct features in its binding/active sites that would account for its preference in the metabolism of certain ligands, such as vincristine [151].

In addition to heme, which is an essential cofactor, CYP3A4 and CYP3A5 require cytochrome b5 and NADPH-cytochrome P450 oxidoreductase as indispensable electron donors/acceptors for optimal catalytic activity [152,153]. Extensive work has considerably elucidated the diverse mechanisms of action that are adopted by the entirety of the CYP superfamily to biotransform their cognate substrates. Some reactions involve C-hydroxylation, epoxide formation, and dealkylation, as well as a host of other reactions, which are reviewed elsewhere [154,155].

2.4.2. Substrates and inhibitors of CYP3A4 and CYP3A5—No distinct substrates that are exclusively metabolized by either CYP3A4 or CYP3A5 are known. Available research data indicate that the two enzymes largely share the same set of substrates/inhibitors but mainly differ in their catalytic efficiency and susceptibility to inhibition [156,157]. Itraconazole, imidazopyridines, and the recently discovered gomisin A are highly selective and much more specific for CYP3A4 than for CYP3A5 [158–160]. In addition, the CYP3A5-catalyzed intrinsic clearance of erythromycin, flunitrazepam, terfenadine, and testosterone is less than 65% than that of CYP3A4 [161]. Conversely, CYP3A5 is the preferential CYP3A subfamily member for metabolizing vincristine and catalyzing midazolam hydroxylation and lidocaine demethylation [151,161,162]. In addition, the catalytic conversion of methyl 2-(4-aminophenyl)-1-oxo-7-(pyridin-2-ylmethoxy)-4-(3,4,5-trimethoxyphenyl)-1,2-dihydroisoquinoline-3-carboxylate into its corresponding N-oxide metabolite is more associated with CYP3A5 than it is with CYP3A4 [163]. CYP3A4 is also reported to be more susceptible than CYP3A5 to inhibition by ketoconazole, fluconazole, nicardipine, diltiazem, and erythromycin [164–166].

3. RELEVANCE OF CYP3A4 AND CYP3A5 TO DRUG DISCOVERY

3.1. Adverse drug–drug interactions

It is common practice to treat diseases by administering combinations of drugs. Hence, a critical step in drug discovery and development involves the assessment of not only the metabolic profile of a drug candidate but also its potential interactions with clinically used drugs. One type of adverse drug–drug interaction (DDI) impairs the activation, metabolism, or elimination of another drug, resulting in increased systemic exposure of the affected drug and a higher risk of toxicity. A notable example is the DDI between terfenadine and CYP3A4 inhibitors, which led to the withdrawal of the former from the market [167–170]. Terfenadine is a prodrug with antihistamine activity that can cause cardiotoxicity. The parent drug can be biotransformed by CYP3A4 to a nontoxic and pharmacologically active metabolite in the liver. However, patients who were taking CYP3A4 inhibitors (e.g. ketoconazole) concurrent with terfenadine reportedly experienced cardiac arrhythmias.

Another adverse consequence of DDIs is the enhanced clearance of therapeutic agents, which decreases the bioavailability and efficacy of the drug(s) and necessitates dose adjustment while monitoring toxicity levels. The acute reduction in plasma concentration of temsirolimus when co-administered with dexamethasone in patients with advanced renal cell carcinoma is an apt illustration of such a DDI [171,172]. Dexamethasone is an established, strong inducer of CYP3A4 and, therefore, reduces the systemic concentration of temsirolimus.

It is also noteworthy that the high rate of genetic polymorphisms that affect CYP3A5 can have great implications for DDIs. Patients with HIV who have the *CYP3A5*1* allele and are undergoing treatment with a combination of darunavir (DRV) and etravirine experience appreciably reduced DRV plasma concentration and efficacy, compared with patients who are homozygous for the *CYP3A5*3* allele [173]. The authors hypothesized that etravirine most likely facilitates the elimination of DVR through PXR- or CAR-dependent induction of *CYP3A5*, which is not expressed in patients who are homozygous for *CYP3A5*3*.

3.2. Benefits of targeting CYP3A4 and CYP3A5 in drug discovery

Drug discovery approaches will benefit from a thorough understanding of the functional roles of CYP3A4, CYP3A5 and other CYPs in different cancers. Presently, much of the knowledge about these enzymes is limited to their xenobiotic response, namely bioactivation of prodrugs and metabolic inactivation of drugs. Tamoxifen, cyclophosphamide, tegafur, and flutamide are some of the many anticancer agents that are administered as prodrugs [174]. Some reasons for designing chemotherapeutic agents as prodrugs include attenuating their cytotoxicity, preventing rapid degradation of their active metabolites, and achieving targeted delivery to the tumor site while sparing nontumor tissues [175,176]. High expression levels of CYP3A4 and CYP3A5 in certain cancers could be exploited to design prodrugs that will only be activated in the cancer cells and promote targeted drug sensitivity.

On the other hand, as discussed in Section 2.1, aberrant expression of CYP3A4 and/or CYP3A5 substantially impacts the progression of breast, colorectal, and pancreatic cancers [9,46,77]. Although high levels of CYP3A4 and CYP3A5 are reported to be associated with

some cases of anticancer drug resistance, the precise mechanism involved is yet to be understood. Recent evidence from studies of exocrine-like PDAC suggests that the response of cancer cells to drugs is severely affected by elevated CYP levels. The key features of exocrine-like PDAC include high basal and drug-induced expression levels of CYP3A5, as well as resistance to erlotinib, paclitaxel, and dasatinib [9]. Inhibition of CYP3A5 activity restores the sensitivity of exocrine-like PDAC cells to tyrosine kinase inhibitors. Therefore, targeting CYP3A5 and its upstream regulators, such as PXR, is a cogent strategy for the management of CYP3A5-mediated cancer drug resistance.

4. CONCLUDING REMARKS

Emerging evidence indicates that the CYP3A4 and CYP3A5 isoforms may be distinguished by their differential expression pattern, enzyme activity, or regulatory mechanisms in cancers and healthy tissues. Therefore, selectively targeting either enzyme or one of their regulators may be an advantageous strategy to overcome CYP3A-mediated cancer drug resistance or adverse DDIs. Adverse drug effects in pharmacotherapy could be alleviated by profiling clinical drugs or developing small-molecule compounds that can selectively modulate the expression or enzymatic activities of CYP3A4 and/or CYP3A5. In addition, libraries of synthetic compounds or natural products (extracts or purified components) can be screened for transcriptional, posttranscriptional or posttranslational regulation of either enzyme.

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Figure 1. CYP3A cluster on Chromosome 7. The gene cluster on 7q21-22 contains four functional genes and two pseudogenes (*CYP3AP1* and *CYP3AP2*). *CYP3A43* is the only gene in the cluster that is in an opposite direction.

CYP3A4_HUMAN	1	MALIPDLAMETWLLLAVALVLLVLYGTHSHGLFKKLGIPGPTPLPFLGNI	50
CYP3A5_HUMAN	1	MDLIPNLAVETWLLLAVALVLLVLYGTRTHGLFKRLGIPGPTPLPFLGNV	50
CYP3A4_HUMAN	51	LSYHKGFCMFDMECHKYGVWGFYDGGQPVLAITDPMIKTVLVKECYS	100
CYP3A5_HUMAN	51	LSYRQGLWKFDTECYKKYGVWGFYEGQLPVLAITDPMVIRTVLVKECYS	100
CYP3A4_HUMAN	101	VFTNRRPFGPVGFMKSAISIAEDEEWKRLRSLSPFTSGKLEKMPPIIA	150
CYP3A5_HUMAN	101	VFTNRRSLGPVGFMKSAISLAEDEEWKRLRSLSPFTSGKLEKMPPIIA	150
CYP3A4_HUMAN	151	QYGDVLRNLRREAETGKPVTLKDVFGAYSMDVITSTSGVNIIDSLNNPQ	200
CYP3A5_HUMAN	151	QYGDVLRNLRREAEGKPVTLKDVFGAYSMDVITGTSGVNIIDSLNNPQ	200
CYP3A4_HUMAN	201	DPFVENTKKLLRFDLDPFFLSITVFPFLIPLEVLNICVFPREVTFNLR	250
CYP3A5_HUMAN	201	DPFVESTKKFLKFGFLDPLFLSIILFPFLTFVFEALNVSLFPKDTINFLS	250
CYP3A4_HUMAN	251	KSVKRMKESRLEDTCQKRVDFLQMLIDSQNSKETESHKALSDLELVAQSI	300
CYP3A5_HUMAN	251	KSVNRMKSRRLNDKQKRVDFLQMLIDSQNSKETESHKALSDLELAAQSI	300
CYP3A4_HUMAN	301	IFIFAGYETTSSVLSFIMYELATHPDVQQLQEEIDAVLPNKAPPTYDTV	350
CYP3A5_HUMAN	301	IFIFAGYETTSSVLSFTLYELATHPDVQQLQKEIDAVLPNKAPPTYDAV	350
CYP3A4_HUMAN	351	LQMEYLDMVVNETLRLFPFIAMRLERVCKKDVINGMFIPKGVVVMIPSYA	400
CYP3A5_HUMAN	351	VQMEYLDMVVNETLRLFPVAIRLERTCKKDVINGVFIKGSVMVVIPTYA	400
CYP3A4_HUMAN	401	LHRDPKYWTEFEKFLPERFSKKKNIDFYIYTPFGSGPRNCIGMRFALM	450
CYP3A5_HUMAN	401	LHHDPKYWTEPEEFRPERFSKK-KDSIDFYIYTPFGTGPNCIGMRFALM	449
CYP3A4_HUMAN	451	NMKLALIRVLQNFSFKPKKETQIPLKLSLGGLLQPEKPVVLKVESRDGTV	500
CYP3A5_HUMAN	450	NMKLALIRVLQNFSFKPKKETQIPLKLDLQGLQPEKPIVLKVDSDGTG	499
CYP3A4_HUMAN	501	SGA	503
		.	
CYP3A5_HUMAN	500	SGE	502

Figure 2. Sequence homology between CYP3A4 (blue) and CYP3A5 (red). The amino acid sequences of both proteins are 84.1% identical. Alignment was done by EMBOSS Pairwise alignment: vertical strokes indicate identical amino acids, colons indicate amino acids with related side chains, and full stops represent amino acids without similarities in their side chains.

Figure 3.

Crystal structures of CYP3A4 and virtual structure of CYP3A5. PyMol was used to depict the crystal structures of free (A) and erythromycin-bound (B) CYP3A4, which were culled from Protein Data Bank with entry numbers 1TQN and 2J0D respectively. The hypothetical structure of ritonavir-bound CYP3A5 (C) was obtained using the molecular simulation methods of Saba *et al.* [144]. Heme is in blue, and ligand in red

Table 1

Expression of CYP3A4 and CYP3A5 in neoplastic and corresponding healthy tissues

Cancerous Tissues	CYP3A4	CYP3A5	Reference
Pancreatic ductal adenocarcinoma (exocrine-like subtype)	–	++	[9]
Hepatocellular	++	±	[57,74]
Breast	++	++	[48,75,76]
Ovarian	++	++	[7]
Colorectal	++	++	[77]
Renal cell	+	+	[36,78]
Lung	–	±, ++	[79]
Prostate	+	±	[50–52,80]

⁺ Similar protein levels in healthy and cancerous cells⁺⁺ Higher level in cancerous cells than in healthy cells[±] Present in healthy cells but downregulated in cancer cells^{±, ++} Different phenotypes caused by polymorphism[–] Absent/not reported in healthy or cancerous cells