Review

Are Orphan Cytochromes P450 Suitable Targets for Breast Cancer Prevention and Treatment?

Barbara Licznerska , Hanna Szaefer , Wanda Baer-Dubowska *

Posted Date: 23 May 2025

doi: 10.20944/preprints202505.1835.v1

Keywords: orphan CYPs 450; CYP4Z1; CYP2S1; CYP2W1; CYP2U1; breast cancer treatment

 doi:10.20944/preprints202505.1835.v1

Review

Are Orphan Cytochromes P450 Suitable Targets for Breast Cancer Prevention and Treatment?

Barbara Licznerska, Hanna Szaefer and Wanda Baer-Dubowska *

Department of Pharmaceutical Biochemistry, Poznan University of Medical Sciences, Poznań, Poland

* Correspondence: baerw@ump.edu.pl; +48 618546624

Abstract: Although significant advances in the treatment of breast cancer have been made over the last decades, searching for more effective prophylaxis and therapy for this type of cancer is still topical. Orphan cytochromes (CYPs) P450 are the enzymes whose functions and substrates are not fully known. The overexpression of some orphan CYPs in breast cancer tissue warrants attention as a possible breast cancer prophylaxis or treatment target. Of particular interest is CYP4Z1, which seems to be specific for breast cancer, including TNBC. The currently available data indicate that inhibition of CYP4Z1 breast-specific expression may reduce the growth, progression, angiogenesis, and invasiveness of breast cancer. Although less specific, the other orphan CYPs, such as CYP2W1, CYP2S1, CYP2U1, and CYP4X1, show much higher expression in breast tumors than in normal tissues. The available data indicate that these CYP isoforms catalyze fatty acids hydroxylation. Their products, such as EETs or HETEs, are considered critical modulators of cancer progression. Therefore, inhibition of the expression and activity of these orphan CYPs might be more useful in cancer treatment than prophylaxis. This review summarizes current knowledge of orphan CYPs in the breast tissue and their possible application in drug targeting.

Keywords: orphan CYPs 450; CYP4Z1; CYP2S1; CYP2W1; CYP2U1; breast cancer treatment

1. Introduction

Cytochromes P450 (CYPs) catalyze a variety of reactions and are of significant importance in the areas of xenobiotics metabolism, including drugs and carcinogens. It is estimated that ~75% of drug metabolism reactions involve CYPs, and ~66% of carcinogens are bioactivated by this class of enzymes [1]. CYP enzymes play an important role in carcinogenesis initiation through the activation of several environmental and endogenous carcinogens, such as estrogen metabolites, as well as in the latter stages of cancer development. Therefore, CYPs are considered promising targets in cancer prophylaxis and chemotherapy. Moreover, they may activate or inactivate prodrugs [2,3]. While most of these reactions involved CYPs with well-characterized function and substrate specificity, it is clear now that some orphan CYPs may be equally important for drugs and carcinogens transformation. The term orphan CYPs was adopted from descriptions of the steroid nuclear superfamily [4] and it is estimated that of the 57 human cytochromes P450 (P450) and 58 pseudogenes discovered to date, 1/4 remain "orphans" in the sense that their function, expression sites, and regulation are still largely not elucidated [5]. Interestingly, the expression of some of these orphan CYPs is tissue-specific and increases in the process of tumorigenesis. For example, CYP4Z1 was found to be frequently upregulated in primary mammary carcinoma and ovarian cancer and was associated with tumor progression and metastasis [6,7]. The others, such as CYP2W1, CYP2S1, CYP2U1, CYP4X1, and CYP4V2, are less specific, but their overexpression in breast cancer has also been found. Attempts are made to deorphanize some of them, but so far only partly successful [8]. Breast cancer is the leading cause of cancer-related deaths among women worldwide. Although breast cancer mortality has been dropping since the 1990s [9], much still has to be done to reduce these numbers. Estrogens play a key role in the pathogenesis of breast cancer. Immunohistochemically, breast cancer has been classified

 doi:10.20944/preprints202505.1835.v1

into four subtypes: estrogen receptor-positive (ER+), progesterone receptor-positive (PR+), human epidermal growth factor receptor 2-positive (HER+), and triple-negative (TNBC) [10]. TNBC accounts for 10-20% of all cases and is resistant to conventional endocrine therapy [11]. Therefore, TNBC has a particularly unmet need for highly effective therapeutic agents.

Thus, the new druggable targets, such as the orphan CYPs expressed in breast epithelial cells, are of particular interest in targeted breast cancer prophylaxis and therapy [12]. This review focuses on the description and discussion of current knowledge of orphan CYPs in breast tissue and their possible application in drug targeting.

2. Expression of Orphan CYPs in Breast Epithelium and Cancer

Most data on the expression of orphan CYPs genes or enzyme activity in the breast epithelium come from in vitro studies using breast cancer cell lines derived from cancer differing in tumor origin and receptor status. In the most extensively investigated ER+ MCF7 breast cancer cell line, the expression of three orphan CYPs, namely CYP4Z1, CYP2S1, and CYP2W1, was confirmed by several authors [13–20]. The analysis of the CYPs expression profile in the ER+ T47D cell line, also derived from ductal carcinoma, revealed the presence of CYP4Z1 [7,16,21,22]. Stable expression of CYP4Z1 was achieved in BT-474 human HER+ breast cancer cells [21]. CYP4Z1 isoform has triggered particular interest because of its hypothetical role in breast cancer through the formation of the signaling molecule 20-hydroxyeicosatetraenoic acid (20-HETE). The expression of this orphan isoform, as well as of CYP2S1 and CYP2W1, was also described in triple-negative cell lines MDA-MB-231 and MDA-MB-468 [13,14,17,18]. Moreover, in comparison to ER+ and PR+ breast cancer cells, higher levels of expression of orphan CYPs were observed in these cell lines. Concurrently, in the non-tumorigenic breast epithelial MCF10A cell line, several orphan CYPs mRNA were detected, in particular CYP2S1, CYP2U1, CYP4V2, CYP4X1, or CYP4Z1. However, CYP4Z1 protein was not revealed on the MCF10A cells' surface [23,24].

To establish the possible role of orphan CYPs in breast cancer development and/or chemotherapy resistance, the group of Tao Xi [17,25,26] analyzed the synergic expression of CYP4Z1 and pseudogene CYP4Z2P in the cells of regular MCF7, tamoxifen-resistant MCF7-TamR, and MDA-MB-231 cell lines. They concluded that overexpression of CYP4Z1 and/or 4Z2P may enhance the transcriptional ERα activity, apoptosis, stemness, and resistance to tamoxifen of breast cancer cells. These observations confirmed the study, which used a human transgenic model, showed that overexpression of CYP4Z1 in lactating female transgenic mice did not result in tumor formation or other mammary abnormalities, but upregulated estrogen receptor (ERα) expression was observed [27]. Additional in vitro studies of this group suggested that human CYP4Z1 might metabolize a small molecule into a transcriptional activator of ERα. Moreover, stable overexpression of CYP4Z1 in breast cancer cells has been reported to promote angiogenesis and tumor growth in mice [21].

Table 1 summarizes the in vitro studies on the orphan CYPs in human breast epithelial cell lines.

Table 1. In vitro studies on the orphan CYPs in human breast epithelial cell lines.

Orphan CYP450	In vitro model (cell line)	Results/Conclusions	References
2S1	MCF10A	mRNA detected predominantly or exclusively in sub-confluent cultures	[23]
	MCF7 MDA-MB-468	- mRNA in both cell lines; higher expression in MDA-MB-468; - low protein level; - expression induced by exogenous AhR ligands	[13]
	MCF7 MDA-MB-231	- mRNA and protein in both cell lines; - higher expression in MDA-MB-231;	[14]

 doi:10.20944/preprints202505.1835.v1

		- increased expression in MCF7 by synthetic methoxystilbes; - decreased mRNA in MDA-MB-231 cells by resveratrol and synthetic methoxystilbes (3MS, 4MS, 5MS)	
2U1	MCF10A	mRNA detected both in sub-confluent and confluent cultures	[23]
2W1	MCF7 MDA-MB-468	- mRNA in both cell lines; - higher expression in MDA-MB-468; - expression induced by exogenous AhR ligands	[13]
	MCF7 MDA-MB-231	- mRNA and protein in both cell lines; - higher expression in MDA-MB-231; - increased protein in MCF7 by synthetic methoxystilbe 3MS; - decreased mRNA in MDA-MB-231 cells by resveratrol and synthetic methoxystilbes (3MS, 4MS, 5MS)	[14]
4V2	MCF10A	mRNA detected both in sub-confluent and confluent cultures	[23]
4X1	MCF10A	mRNA detected both in sub-confluent and confluent cultures	[23]
4Z1	MCF7	mRNA and protein in breast tissue (normal and cancer) with low expression levels (in comparison to other human CYPs) in other tissues (e.g., liver)	[15]
	MCF7 T47D	- mRNA preferentially expressed in mammary tissue; - implication of PG and glucocorticoid receptor in CYP4Z1 gene activation	[16]
	MCF10A	mRNA detected both in sub-confluent and confluent cultures	[23]
	T47D BT-474	immunostaining overexpression promotes tumor angiogenesis and growth in breast cancer	[21]
	MCF7 and MCF7-TamR	- CYP4Z1 and CYP4Z2P downregulated in MCF7 compared with MCF7-TamR; - overexpression of CYP4Z1- or CYP4Z2P-3′UTR enhances the transcriptional activity of ERα; - the blocking of CYP4Z1- and CYP4Z2P-3′UTR reversed tamoxifen resistance in MCF7-TamR	[25]
	MCF7 MDA-MB-231	downregulation of CYP4Z1- or CYP4Z2P-3′UTR promotes cell apoptosis	[26]
	MCF7 MDA-MB-231	the comprehensive endogenous RNA network mediated by CYP4Z1 gene and CYP4Z2P pseudogene promoted the stemness of breast cancer	[17]
	MCF7 MDA-MB-231	overexpression of CYP4Z1 3′UTR could suppress the capacity of migration and adhesion of these cells via acting as	[18]

 doi:10.20944/preprints202505.1835.v1

		competitive endogenous RNAs for E-cadherin	
	MCF7	- demonstrate the presence of CYP4Z1 enzyme on the outer surface of the plasma membrane of MCF7; - the detection of high titers of anti-CYP4Z1 aAbs in breast cancer patients but not in healthy controls	[19]
	MCF10A	no display of CYP4Z1 on MCF10A cells surface	[24]
	T47D transfected with CYP4Z1	inhibition of 14,15-EET (product of arachidonic acid metabolism influencing proliferation, migration, and angiogenesis) via designed synthetic '7' inhibitor	[22]
	combination of in vitro and in silico models of recombinant CYP4Z1 mutants	Arg487 and Asn381 residues in CYP4Z1 protein play a crucial role in substrate recognition and binding	[28]
	MCF7 MDA-MB-231	- confirming of HET0016 as a synthetic CYP4Z1 inhibitor; - CYP4Z1 promoted the stemness of MCF7 breast cancer cells	[29]
	MCF7	discovery of the novel CYP4Z1 inhibitors in the enzyme bags test and the CYP4Z1-overexpressing MCF7 cell clone	[30]
	MCF7 BT549 SUM159 MDA-MB-231	- CYP4Z1 mRNA expression - 20-HETE treatment promoted the growth of TNBC cell lines (BT549, SUM159, MDA-MB-231)	[20]

A more recent clinical study showed a high incidence of CYP4Z1 expression in TNBC patients with advanced grades, later stages, and larger tumors [31]. Clinical studies of the other orphan CYPs expression profile and its relationship with clinical pathological variables, although limited, showed an interesting trend. Murray et al. [6] performed immunostaining of a tissue microarray containing 170 breast cancers of no special type for a panel of 21 CYPs.

The highest percentage of strong immunopositivity in these samples was seen for CYP4X1, CYP2S1, and CYP2U1. At the same time, CYP4V2, CYP4X1, and CYP4Z1 showed correlation with the tumor grade. Association with survival was identified for CYP2S1, CYP3A4, CYP4V2, and CYP26A1, although none of these P450s were an independent prognosis biomarker [6]. In some smaller pools of patients, the increased transcript levels of CYP2S1, CYP2W1, and CYP4F11 both in breast cancer, adjacent, and normal breast cells were found. Unfortunately, protein levels were inappropriately low to confirm the results [32,33]. Table 2 presents the results of the orphan CYPs analyses in clinical samples.

Table 2. Clinical studies on the orphan CYPs in breast cancer patients.

Orphan CYP450	**patients pool**	**Results/Conclusions**	**References**
2A7	20 tumor and control breast tissue samples	no mRNA detected	[34]
	165 triple-negative-breast-cancer samples	expression associated with poorer survival	[35]

 doi:10.20944/preprints202505.1835.v1

2S1	170 breast cancer, no special type samples	- 37,5% of CYP2S1 immune-positive cells; - the absence of CYP2S1 correlated with better survival	[6]
	50 breast cancer patients and 31 controls	- mRNA increased; - protein not detected	[32]
	1,426 early-stage invasive breast cancer	- low immunohistochemical protein expression associated with poorer patient survival	[36]
2U1	170 breast cancer, no special type samples	- 32,2% of CYP2S1 immune-positive cells; - correlated with tumor grade	[6]
	219 invasive breast cancer	- high immunohistochemical protein level correlated with poorer survival - more frequent in TNBC	[37]
2W1	32 breast cancer patients and 20 controls	- mRNA expressed in breast cancer, adjacent, and normal breast cells; - 230x higher in breast cancer than in normal breast cells; - expression associated with Ki67	[33]
	50 breast cancer patients and 31 controls	- significantly overexpressed in tumors; - higher 2W1 mRNA correlated with a better response to neoadjuvant chemotherapy; - not confirmed on protein level (too low)	[32]
	1,426 early-stage invasive breast cancer	- low immunohistochemical protein expression associated with poorer patient survival	[36]
3A43	170 breast cancer, no special type samples	70,7% of samples most frequently displayed no immunoreactivity	[6]
	1,143 incident breast cancer cases and 1155 population controls	allele CYP3A43_74_delA correlated with higher grade breast tumors	[38]
4F11	32 breast cancer patients and 20 controls	- mRNA expressed in breast cancer, adjacent, and normal breast cells; - no statistical differences between cancer and normal tissue; - expression associated with Ki67	[33]
4V2	170 breast cancer, no special type samples	- immunostaining was correlated with survival; - correlated with tumor grade	[6]
4X1	ONE individual patient	mRNA detected	[39]
	170 breast cancer no special type samples	- 50,8% of CYP4X1 immune-positive cells; - immunostaining correlated with lower tumor grade	[6]
	120 primary breast cancer and 5 nontumorigenic controls	off-frame fusion with pseudogene CYP4Z2P of unknown function	[40]
	105 breast cancer patients with neoadjuvant cytotoxic chemotherapy	Variant rs17102977 in CYP4X1 associated with response to neoadjuvant cytotoxic chemotherapy	[41]
4Z1	54 breast tumors	mRNA overexpression (microarray) in ~ 50% of samples	[42]

 doi:10.20944/preprints202505.1835.v1

	170 breast cancer, no special type samples	immunostaining correlated with increasing tumor grade	[6]
	249 breast cancer patients ER+	- immunostaining correlation between mutated oncogene *PIK3CA* and overexpression of CYP4Z1 and pseudogene CYP4Z2P	[43]
	paraffin-embedded breast cancer tissue samples and 8 pairs of fresh breast cancer and normal tissues	the comprehensive endogenous RNA network mediated by the CYP4Z1 gene and the CYP4Z2P pseudogene promoted the stemness of breast cancer	[17]
	???	overexpression of CYP4Z1 3′UTR could suppress the capacity of migration and adhesion of these cells by acting as competitive endogenous RNAs for E-cadherin	[18]
	sera from 19 breast cancer patients 11 control sera	- demonstrate the presence of CYP4Z1 enzyme on the plasma membrane of MCF7; - the detection of high titers of anti-CYP4Z1 aAbs in breast cancer patients but not in healthy controls	[19]
	220 breast cancer cases 8 normal breast tissues	- immunohistochemically, 82% of malignant samples with a moderate-intense expression; - normal tissues and benign tumors: no to weak expression	[44]
	122 TNBC cases 4 normal breast tissues	- a strong expression of CYP4Z1 (83,3%) in various TNBC subtypes - negative expression in normal samples - poorer overall survival of TNBC patients with high CYP4Z1 expression in comparison to patients with low CYP4Z1 expression	[31]
	5 TNBC patients	patient-derived xenografts expressed CYP4Z1 mRNA	[20]
	86-anthracycline responsive breast cancer patients vs. 7-anthracycline non-responsive	CYP4Z1 significantly upregulated in the anthracycline-resistant group	[45]

As it was mentioned above, much attention is focused on the role of CYP4Z1 and an associated pseudogene, CYP4Z2P, because of their breast-epithelium-specific expression. In this regard, Cizkova et al. [43] noticed in the series of 249 ER+ breast cancer patients a correlation between the mutation status of oncogene *PIK3CA* and overexpression of these CYPs.

Finally, the expression of CYP2A7, an orphan CYPs of so far smaller interest in the context of breast cancer, showed an association with poorer survival of TNBC patients [35].

 doi:10.20944/preprints202505.1835.v1

3. Orphans CYPs as the Therapeutic Targets - Mechanistic Studies

As was described in the previous section, several orphan CYPs were detected in breast epithelium. Here, the most studied isoforms' characteristics and their potential for prevention or therapeutic purposes are provided.

3.1. CYP4Z1

Some experimental data, including the analysis of Human Protein Atlas for CYP4Z1 expression in normal and cancer tissues, confirm that CYP2Z1 is preferentially localized in the breast and its activity is dramatically increased in breast cancer [7]. Therefore, modulation of CYP2Z1 activity appears to be an interesting target, even a kind of "silver bullet" for breast cancer treatment [46]. The CYP4Z1 gene is located on chromosome 1p33, in a cluster containing the CYP4A11 and CYP4X1 genes. CYP4Z1 shares 54% and 52% sequence identity with CYP4X1 and CYP4A11, respectively [16]. Comparative genomics analysis demonstrated that tumor-specifically expressed sequences, such as CYP4Z1, are either evolutionarily new (primates or humans) or relatively young (mammals) [47]. Consequently, no orthologs have been found in mice or rats, so CYP4Z1 appears to be specific to humans and primates [48], which, to some extent, limits the investigation of animal models. On the other hand enables to creation of human transgenic models mentioned in the previous section. Designing suitable small molecules as potential drugs or chemopreventive agents requires knowledge of the metabolically based role of CYP4Z1 in breast cancer progression and identifying its substrates.

In this context, it is worth noticing that in contrast to the other members of the CYP4 family, CYP4Z1 characterizes the presence of heme not covalently linked [49].

The first functional study of CYP4Z1 was performed in 2009 by Bureik's research group. Using human CYP4Z1 expressed in yeast, they found a unique pattern of metabolites with lauric acid generating mainly the 8-hydroxy (ω-4) product and myristic acid forming 12-hydroxy (ω-2) [50].

Subsequent studies focused on the possibility of generation by CYP4Z1 of arachidonic acid metabolites such as pro-angiogenic epoxyeicosatrienoic acids (EETs) and HETE acids considered critical modulators of cancer progression, acting in concert with endothelial growth factor and others growth factors ultimately promoting cellular proliferation, neovascularization, angiogenesis, and metastasis. Of particular interest is 20-HETE, which, as it was postulated, is responsible for proliferative, angiogenic, and tumor growth effects [21].

However, CYP4Z1 is considered an EET synthase. Therefore, it was not surprising that the major products of internal oxidation of arachidonic acid (AA) in breast ductal carcinoma T47D cells engineered to express CYP4Z1 were derivatives 14,15-EET and 14,15-dihydroxy eicosatrienoic acid. Only a trace of 20-HETE was detected, leading to conclusions that CYP4Z1 is distinct from other CYP4 enzymes and 20-HETE is not the major AA metabolite produced in a reaction catalyzed by CYP4Z1 [7,22].

Besides, fatty acids screening for the other substrates was performed in yeast enzyme bags (permeabilized cells from recombinant fission yeast cells) and indicated the ability of CYP4Z1 to catalyze 11-*O* -dealkylation cleavages and 2-hydroxylation reactions of pro-luciferin compounds [7,29,51]. Through docking experiments and site-directed mutagenesis, Asn381 and Arg487 were pointed out as key active sites of CYP4Z1 [7].

The subsequent search for inhibitors was based on the fatty acid hydroxylase activity of CYP4Z1. One of the tested compounds was N-hydroxy-N'-(-butyl-2-methylphenyl)-formamidine (HET0016), a highly potent inhibitor of 20-HETE synthase. Although the results of initial studies [21] were promising, further investigations indicated only weak inhibition of CYP4Z1 [52]. It was in contrast to results obtained with the other members of the CYP4 family and might be related to their covalent linkage with heme. The first selective mechanism-based CYP4Z1 inhibitor was discovered by Kowalski et al. in 2020, namely 8-[(1H-benzotriazol-1-y)amino]octanoic acid [22]. Analysis of major metabolites of this compound in rats' plasma showed that products of β-oxidation, with a probable

 doi:10.20944/preprints202505.1835.v1

higher inhibitory effect than the parent compound [7]. One of the latest proposed CYP4Z1 inhibitors is a derivative of HET0016 with an esterified 4-carbon carboxylate tail [53]. This compound, tested in MCF7 and MDA-MB-231 cells, showed the ability to reduce metastatic potential, spheroid formation, and expression of stemness markers. Low constitutive expression of CYP4Z1 in these cell lines requires careful interpretation of these data, although even minimal activity of this enzyme might be sufficient to obtain the desired effect [7]. Previously, Rieger et al. [15] suggested utilizing the tissue and cancer-specific expression of CYP4Z1 to bioactivate prodrugs into active agents for breast carcinoma treatment. However, despite attempts, no fruitful results have been obtained so far that would allow the use of CYP4Z1 activity to activate antibreast cancer prodrugs.

The observation that MCF7 breast cancer cells, in contrast to non-tumorigenic MCF10A cells, display CYP4Z1 on their surface might indicate its usefulness in breast cancer immunotherapy [24].

3.2. CYP2S1

As was mentioned in section 2, orphan CYP2S1 was reported to be upregulated in breast cancer cells and clinical samples. Moreover, its association with patient survival was found [36]. However, CYP2S1, in contrast to CYP4Z1, is not unequivocally linked with this tissue. Therefore, the data on its usefulness as a drug in breast cancer was not extensively studied.

The human CYP2S1 gene is located on chromosome 19, at the 19q13.2 region, encodes a protein of 504 amino acids [54], and is mainly expressed in the endoplasmic reticulum. The highest expression of CYP2S1 was found in the epithelium of portal entry organs, but the lower expression was described in several organs, including breast epithelium. CYP2S1 catalyzes the oxidation of AA into 19-hydroxy-5Z,8Z, 11Z, and 14Z-eicosatetraenoic acid, among other polyunsaturated fatty acids (PUFA) with ω-1-PUFA [55]. CYP2S1 also possesses epoxygenase activity involved in the metabolism of prostaglandins, modulating the inflammatory process [56].

As CYP1 family members, AhR and ARNT regulate the induction of CYP2S1 [57]. Moreover, CYP2S1 peroxidase activity linked this CYP isoform with the metabolism of xenobiotics, such as environmental toxins/pollution and small-molecule drugs [58]. CYP2S1 can activate some anticancer prodrugs, e.g., ellipticine to 12-hydroxyellipticine and 13-hydroxyellipticine [8,59–61]. Therefore might be considered a more general therapeutic target. Analysis of the antitumor activity of GW-610 (2-(3,4-dimethoxyphenyl)-5-fluorobenzothazole) and 5F-203 (2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole) in breast cancer cell lines selectively depleted of CYP1A1, CYP2S1, and CYP2W1 showed that in contrast to CYP1A1 and CYP2W1, CYP2S1 mediates these drugs inactivation [13,62]. More detailed analysis of the metabolites formed from these drugs suggested that in its activation-deactivation hydroxylamine metabolite is involved, which can either be reduced back to the parent compound by CYP2S1 or progress to the formation of DNA adducts, mainly dGuo [62].

As was mentioned in the previous section, CYP2S1 expression was found in breast cancer cells differing in hormone receptors status. Interestingly, treatment of these cells with some methoxy stilbenes or resveratrol increased the expression of CYP2S1 in ER+ MCF7 cells, but not in ER- MDA-MB-231, where a decrease of the favorable level of this CYP was observed [14].

3.3. CYP2W1

Similarly to CYP2S1, the expression of CYP2W1 was described in breast cancer cells differing in hormone receptors status, and its upregulation was found in breast cancer samples [36]. Moreover, cells with higher levels of expression of these orphan CYPs in comparison to ER+ and PR+ breast cancer cells were observed in MDA-MB-231 and MDA-MB-468 cells.

The CYP2W1 gene is located on chromosome 7 p22.3 and encodes a protein comprising 490 amino acids [63]. The highest expression of CYP2W1 was noticed in the prostate and pancreas. The CYP2W1 expression is, to a great extent, regulated through epigenetic mechanisms mainly by DNA methylation. DNA hypermethylation is a pivotal epigenetic mechanism that silences many genes, including those regulating cell cycle, inflammation, stress response, DNA repair, and apoptosis [64].

 doi:10.20944/preprints202505.1835.v1

Hypermethylation of certain genes, particularly tumor suppressor genes, is known to be associated with the inactivation of various pathways involved in tumorigenesis. CYP2W1 gene expression is supposed to be regulated by epigenetic modification, namely DNA methylation of its promoter CpG islands. In this regard, the early study of Gomez et al. [65] showed that the expression of CYP2W1 in colon cancer was associated with the methylation status of its promoter and suggested a causal link between the gene CpG island demethylation and CYP2W1 enhanced expression. This suggestion is further supported by the fact that CYP2W1 is expressed in the course of development of the gastrointestinal tract, silenced after birth in the intestine and colon by its promoter CpG island hypermethylation, but activated following demethylation. Therefore, it seems that demethylation is a prerequisite for CYP2W1 expression, probably also in breast cancer cells [65].

CYP2W1 is induced by AhR and ARNT pathway, as shown in the experiments in which treatment of MDA-MB-468 and MCF7 breast cancer cells with AhR ligands such as 5F-203 and GW-610 increased expression of CYP2W1 [13,66]. Moreover, in contrast to CYP2S1, CYP2W1 was involved in the activation of these drugs [6].

Untargeted substrate searches showed that CYP2W1 catalyzes both hydroxylation and epoxidation of several fatty acids and phosphatidylcholine [68]. Moreover, among the numerous other endogenous substrates were also steroids, including 17β-estradiol. However, its binding to CYP2W1 was significantly weaker than that of retinoids [69]. CYP2W1 is involved in drug metabolism, facilitating such reactions like N-demethylation and aromatic hydroxylation e.g., benzphetamine [70]. High expression of CYP2W1 in tumor tissue, particularly in breast cancer (overexpression ~230 fold) in comparison to normal mammary gland tissues [33], makes this CYP isoform an attractive anticancer drug target. CYP2W1 having the ability to activate prodrugs can increase the selectivity of chemotherapeutic agents to prevent tumor growth or metastasis as well as reduce the tumor volume before surgery [71]. Besides, CYP2W1 can be a potential target for immunotherapy, since it is located on the cell membrane surface. A specific antibody, derived from the peptide sequence of CYP2W1, was already developed and tested in MCF7 breast cancer cells [72].

3.4. CYP2U1

Immunohistochemical analysis, along with survival analysis based on clinical-pathological features, showed that CYP2U1 is engaged in the malignant progression of breast carcinoma.

Interestingly, CYP2U1 protein level was inversely linked with the state of ER, i.e., much higher in ER- in comparison with ER+ cancer tissue [37]. Therefore, it might be considered another druggable target for the treatment of advanced breast cancer. The human CYP2U1 gene is located on chromosome 4q25. Its protein product comprises 544 amino acids with the region containing 8 proline residues before the transmembrane helix and an insert of about 20 amino acids rich in arginine residues located after the transmembrane helix [73]. CYP2U1 is the only member of this subfamily, and it seems to be very old and highly conserved across species [74]. Similarly to the orphan CYPs described above, CYP2U1 catalyzes fatty acids hydroxylation. These include AA, docosahexaenoic acid, and eicosapentaenoic acids. CYP2U1-mediated metabolism of AA leads to the formation of 19- and 20-HETE. It was also shown that CYP2U1 efficiently catalyzes the hydroxylation of leukotriene B4 (LTB4) predominantly on its ω-position. The involvement of CYP2U1 in the metabolism of LTB4 could have significant physiologic consequences, as LTB4 is an important inflammatory mediator involved in the pathogenesis of many diseases, including cancer [75]. Specific inhibitors of CYP ω-hydroxylases (e.g., 17-octadecenoic acid) decrease CYP2U1-mediated activity [37].

3.5. CYP4X1

As was mentioned in the previous section, CYP4X1, along with the described above orphan CYPs, showed the highest expression in breast cancer samples and correlation with the tumor grade [6].

 doi:10.20944/preprints202505.1835.v1

The CYP4X1 gene is located in the cytochrome P450 ABXZ gene cluster along with CYP4Z2, and according to recent studies, this enzyme catalyzes epoxidation of endogenous cannabinoid anandamide and arachidonic acid [39,76]. The lower gene expression of CYP4X1 was associated with shorter overall survival of Chinese gastric cancer patients treated with capecitabine and oxaliplatin [77].

A recent study by Hlavac et al. [2021] pointed out the association of a specific variant, rs17102977 in the CYP4X1 gene, with the response of breast cancer patients to the neoadjuvant cytotoxic chemotherapy. The substitution rs17102977 in *CYP4X1* intron was associated with both the response of the patients to the neoadjuvant cytotoxic therapy and the disease-free survival of hormonally treated patients. It is also prognostic in patients unselected according to the therapy. The endocannabinoid system is involved in various physiological processes, including inflammation, immunomodulation, and suppression of different cancers, including breast cancer, thus, CYP4X1 may play a role in the response to anticancer chemotherapy via physiological processes. The role of rs17102977 in cancer is, however, unknown [41].

4. Conclusions and Future Direction

Most of the orphan CYPs described in this review seem to be expressed in different cancer tissues, and only one, i.e., CYP4Z1, could be named specifically for the breast epithelium. Therefore, this CYP isoform may be considered the most promising for the treatment of breast cancer. The currently available data indicate that inhibition of CYP4Z1 breast-specific expression may reduce the growth, progression, angiogenesis, and invasiveness of breast cancer. Moreover, the knowledge of the biological mechanism of CYP4Z1 activation could serve for the design of prodrugs activated explicitly to the active drug in the neoplastic tissue of the breast gland, reducing the potential systemic side effects of chemotherapy. Furthermore, a potency to reverse TAM-resistance in CYP4Z1-positive breast cancer cells may be an important tool to enhance the efficacy of existing adjuvant therapies. Finally, the generation of CYP4Z1 antibodies on the surface of breast cancer cells may contribute to the invention of effective immunotherapy for this cancer, employing anti-cancer vaccines. Since CYP4Z1 facilitates breast cancer development by induction of ERα expression, its inhibition may have a double effect, eliminating the major breast cancer risk factor.

CYP2W1, CYP2S1, CYP2U1, and CYP4X1, although not as specific for breast epithelium as CYP4Z1 is, show much higher expression in tumors than in normal tissues. Therefore, they may be considered more general cancer therapeutic targets. The most promising are the results of the studies confirming the importance of these CYPs in TNBC cases, for which treatment options are limited, and the survival prognosis is poorer.

Although the functions and substrates of these CYPs are still not known, it seems that all catalyze fatty acids hydroxylation. Their products, such as EETs or HETEs, are considered critical modulators of cancer progression involved in promoting cellular proliferation, neovascularization, angiogenesis, and metastasis. Therefore, inhibition of the expression and activity of these orphan CYPs might be more useful in cancer treatment than prophylaxis. On the other hand, CYP4Z1 induction of ERα expression may affect tumorigenesis initiation, thus, its inhibition may prevent tumor development.

Unfortunately, the knowledge about orphan CYPs and their role in breast cancer is limited to cell cultures and clinical research with a relatively small pool of patients. The identification of endogenous ligands of these isoforms is still topical and seems to be crucial in understanding their role in breast epithelium homeostasis. The future scope depicts a great need for a broader panel of cell lines as well as clinical trials with large numbers of patients with a reliable non-cancer base. Conceptual studies involving the simultaneous assessment of orphan CYPs transcript and protein levels and the presence of antibodies on the surface of both normal and cancerous breast epithelial cells would be significant.

In conclusion, orphan CYPs expressed both in tumor and non-tumor breast tissues may serve as potential targets for inhibition of tumorigenesis, particularly the delay of progression of breast cancer.

 doi:10.20944/preprints202505.1835.v1

Moreover, the orphan CYPs isoforms influencing the activation of potential prodrugs and sensitizing cells resistant to adjuvant therapy are hopes for effective prevention and treatment of breast cancer.

Author Contributions: Conceptualization, B.L., H.S. and W.B.-D.; writing-original draft preparation, B.L. and H.S.; writing-review and editing, B.L., H.S., and W.B.-D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AA	arachidonic acid
CYPs	cytochromes P450
EET	epoxyeicosatrienoic acid
ER	estrogen receptor
HETE	hydroxyeicosatetraenoic acid
HER	human epidermal growth factor receptor 2
LTB4	leukotriene B4
PR	progesterone receptor
PUFA	polyunsaturated fatty acids
TAM	tamoksifen
TNBC	Triple-negative breast cancer

References

1. Mokhosoev, I.M.; Astakhov, D.V.; Terentiev, A.A.; Moldogazieva, N.T. Human Cytochrome P450 Cancer-Related Metabolic Activities and Gene Polymorphisms: A Review. *Cells* **2024,** *13*, 1958. doi: 10.3390/cells13231958.
2. Nelson DR. A world of cytochrome P450s. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **2013**,*368*, 20120430. doi: 10.1098/rstb.2012.0430.
3. Nelson D.R. Cytochrome P450 diversity in the tree of life. *Biochim. Biophys. Acta Proteins Proteom*. **2018**, *1866*, 141-154. doi: 10.1016/j.bbapap.2017.05.003.
4. Mangelsdorf, D.J.; Evans, R.M. The RXR heterodimers and orphan receptors. *Cell* **1995**, *83(6)*, 841-50. doi: 10.1016/0092-8674(95)90200-7.
5. Guengerich, F.P.; Cheng, Q. Orphan in the human cytochrome P450 superfamily: approaches to discovering functions and relevance in pharmacology. *Pharmacol. Rev.* 2011, *63*, 684-99. doi: 10.1124/pr.110.003525.
6. Murray, G.I.; Patimalla, S.; Stewart, K.N.; Miller, I.D.; Heys, S.D. Profiling the expression of cytochrome P450 in breast cancer. *Histopathology* **2010**, *57*, 202-11. doi: 10.1111/j.1365-2559.2010.03606.x.
7. Kowalski, J.P.; Rettie, A.E. There and Back Again: A Perspective on 20 Years of CYP4Z1. *Drug Metab. Dispos*. **2024**, *52*, 498-507.doi: 10.1124/dmd.124.001670.
8. Molina-Ortiz, D.; Torres-Zárate, C.; Santes-Palacios, R. Human Orphan Cytochromes P450: An Update. *Curr. Drug. Metab.* **2022**, *23*, 942-963. doi: 10.2174/1389200224666221209153032.
9. Torre, L.A.; Bray, F.; Siegel, R.L.; Ferlay, J.; Lortet-Tieulent, J.; Jemal, A. Global cancer statistics, 2012. *CA Cancer J. Clin.* **2015**, *65*, 87-108. doi: 10.3322/caac.21262.

 doi:10.20944/preprints202505.1835.v1

10. Harbeck, N.; Penault-Llorca, F.; Cortes, J.; Gnant, M.; Houssami, N.; Poortmans, P.; Ruddy, K.; Tsang, J.; Cardoso, F. Breast cancer. *Nat. Rev. Dis. Primers* **2019**, *5*, 66. doi: 10.1038/s41572-019-0111-2.
11. Kumar, P.; Aggarwal, R. An overview of triple-negative breast cancer. *Arch. Gynecol. Obstet.* **2016**, *293*, 247-69. doi: 10.1007/s00404-015-3859-y.
12. Licznerska, B.; Baer-Dubowska, W. What might the presence of 'orphan' CYP450 isoforms in breast epithelial cells mean for the future of targeted therapeutics? *Expert Opin. Drug Metab. Toxicol.* **2021**, *17*, 135-137. doi: 10.1080/17425255.2021.1844182.
13. Tan, B.S.; Tiong, K.H.; Muruhadas, A.; Randhawa,N.; Choo,H.L.; Bradshaw,T.D.; Stevens,M.F.G.; Leong Ch.-O. CYP2S1 and CYP2W1 mediate 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (GW-610, NSC 721648) sensitivity in breast and colorectal cancer cells. *Mol. Cancer Ther.* **2011**, *10*, 1982-92. doi: 10.1158/1535-7163.MCT-11-0391.
14. Szaefer, H.; Licznerska, B.; Cykowiak, M.; Baer-Dubowska W. Expression of CYP2S1 and CYP2W1 in breast cancer epithelial cells and modulation of their expression by synthetic methoxy stilbenes. *Pharmacol. Rep.* **2019**, *71*, 1001-1005. doi: 10.1016/j.pharep.2019.08.005.
15. Rieger, M.A.; Ebner, R.; Bell, D.R.; Kiessling, A.; Rohayem, J.; Schmitz, M.; Temme, A.; Rieber, E.P.; Weigle, B. Identification of a novel mammary-restricted cytochrome P450, CYP4Z1, with overexpression in breast carcinoma. *Cancer Res.* **2004**, *64*,:2357-64. doi: 10.1158/0008-5472.can-03-0849.
16. Savas, U.; Hsu, M.-H.; Griffin, K.-J.; Bell, D.R.; Johnson, E.F. Conditional regulation of the human CYP4X1 and CYP4Z1 genes. *Arch. Biochem. Biophys.* **2005**, 436, 377-85. doi: 10.1016/j.abb.2005.02.022.
17. Zheng, L;. Guo, Q.; Xiang, C.; Liu, S.; Jiang, Y.; Gao, L.; Ni, H.; Wang, T.; Zhao, Q.; Liu, H.; Xing, Y.; Wang,Y.; Li, X.; Xi, T. Transcriptional factor six2 promotes the competitive endogenous RNA network between CYP4Z1 and pseudogene CYP4Z2P responsible for maintaining the stemness of breast cancer cells. *J. Hematol. Oncol.* **2019**, 12, 23. doi: 10.1186/s13045-019-0697-6.
18. Wang, B.; Zheng, L.; Chou, J.; Li, C.; Zhang, Y.; Meng, X.; Xi, T.; CYP4Z1 3'UTR represses migration of human breast cancer cells. *Biochem. Biophys. Res. Commun.* **2016**, *478*, 900-7. doi: 10.1016/j.bbrc.2016.08.048.
19. Nunna, V.; Jalal, N.; Bureik, M. Anti-CYP4Z1 autoantibodies detected in breast cancer patients. *Cell. Mol. Immunol.* **2017**, *14*, 572-574. doi: 10.1038/cmi.2017.21.
20. Wu, W.; Warner, M.; Wang, L.; He, W.W.; Zhao, R.; Guan, X.; Botero, C.; Huang, B.; Ion, C.; Coombes, C.; Gustafsson, JA. Drivers and suppressors of triple-negative breast cancer. *Proc. Natl. Acad. Sci. U S A.* **2021**, *118*, e2104162118. doi: 10.1073/pnas.2104162118.
21. Yu, W.; Chai, H.; Li, Y.; Zhao, H.; Xie, X.; Zheng, H.; Wang, C.; Wang, X., Yang, G.; Cai, X.; Falck, J.R.; Yang, J. Increased expression of CYP4Z1 promotes tumor angiogenesis and growth in human breast cancer. *Toxicol. Appl. Pharmacol.* **2012**, *264*, 73-83. doi: 10.1016/j.taap.2012.07.019.
22. Kowalski, J.P.; McDonald, M.G.; Pelletier, R.D.; Hanenberg, H.; Wiek, C.; Rettie, A.E. Design and Characterization of the First Selective and Potent Mechanism-Based Inhibitor of Cytochrome P450 4Z1. *J. Med. Chem.* **2020**, *63*, 4824-4836. doi: 10.1021/acs.jmedchem.0c00101.
23. Thomas, R.D.; Green, M.R.; Wilson, C.; Weckle, A.L.; Duanmu, Z.; Kocarek, T.A.; Runge-Morris, M. Cytochrome P450 expression and metabolic activation of cooked food mutagen 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) in MCF10A breast epithelial cells. *Chem Biol Interact*. **2006**, *160*, 204-16.doi: 10.1016/j.cbi.2006.01.007.
24. Khayeka-Wandabwa, C.; Ma, X.; Cao, X.; Nunna, V.; Pathak, J.L.; Bernhardt, R.; Cai, P.; Bureik, M. Plasma membrane localization of CYP4Z1 and CYP19A1 and the detection of anti-CYP19A1 autoantibodies in humans. *Int. Immunopharmacol.* **2019**, *73*, 64-71. doi: 10.1016/j.intimp.2019.05.003.
25. Zheng, L.; Li, X.; Meng, X.; Chou, J.; Hu, J.; Zhang, F.; Zhang, Z.; Xing, Y.; Liu, Y.; Xi, T. Competing endogenous RNA networks of CYP4Z1 and pseudogene CYP4Z2P confer tamoxifen resistance in breast cancer. *Mol. Cell. Endocrinol.* **2016**, *427*. 133-42. doi: 10.1016/j.mce.2016.03.012.
26. Li, C.; Zheng, L.; Xin, Y; Tan, Z.; Zhang, Y.; Meng, X.; Wang, Z.; Xi, T. The competing endogenous RNA network of CYP4Z1 and pseudogene CYP4Z2P exerts an anti-apoptotic function in breast cancer. *FEBS Lett.* **2017**, *591*, 991-1000.doi: 10.1002/1873-3468.12608.

 doi:10.20944/preprints202505.1835.v1

27. Khayeka-Wandabwa, C.; Zhao, J.; Pathak, J.L.; Wu, H.; Bureik, M. Upregulation of estrogen receptor alpha (ERα) expression in transgenic mice expressing human CYP4Z1. *Breast Cancer Res. Treat.* **2022**, *191*,319-326. doi: 10.1007/s10549-021-06435-w.
28. Du, W.; Machalz, D.; Yan, Q.; Sorensen, E.J.; Wolber, G.; Bureik, M. Importance of asparagine-381 and arginine-487 for substrate recognition in CYP4Z1. *Biochem. Pharmacol.* **2020**, *174*, 113850. doi: 10.1016/j.bcp.2020.113850.
29. Liu, J.; Machalz,D.; Wolber, G.; Sorensen, EJ.; Bureik, M. New Proluciferin Substrates for Human CYP4 Family Enzymes. *Appl. Biochem. Biotechnol.* **2021**, *193*, 218-237.doi: 10.1007/s12010-020-03388-6.
30. Machalz, D.; Li, H.; Du, W.; Sharma, S.; Liu, S.; Bureik, M.; Wolber, G. Discovery of a novel potent cytochrome P450 CYP4Z1 inhibitor. *Eur. J. Med. Chem.* **2021**, *215*, 113255. doi: 10.1016/j.ejmech.2021.113255.
31. Al-Saraireh, Y.M.; Alshammari, F.O.F.O.; Youssef, A.M.M.; Al-Tarawneh, F.; Al-Sarayreh, S.; Almuhaisen, G.H.; Satari, A.O.; Al-Shuneigat, J.; Alrawashdeh, H.M. Cytochrome 4Z1 Expression is Associated with Unfavorable Survival in Triple-Negative Breast Cancers. *Breast Cancer (Dove Med Press).* **2021**, *13*, 565-574.doi: 10.2147/BCTT.S329770.
32. Hlaváč, V.; Brynychová, V.; Václavíková, R.; Ehrlichová, M.; Vrána, D.; Pecha, V.; Trnková, M.; Kodet, R.; Mrhalová, J.; Kubáčková, K.; Gatěk, J.; Vážan, P.; Souček, P. The role of cytochromes p450 and aldo-keto reductases in prognosis of breast carcinoma patients *Medicine (Baltimore).* **2014**, *93*, e255. doi: 10.1097/MD.0000000000000255
33. Bandala, C.; Floriano-Sánchez, E.; Cárdenas-Rodríguez, N.; López-Cruz, J.; Lara-Padilla, E. RNA expression of cytochrome P450 in Mexican women with breast cancer. *Asian Pac. J. Cancer Prev.* **2012**, *13*, 2647-53. doi: 10.7314/apjcp.2012.13.6.2647.
34. Iscan, M.; Klaavuniemi, T.; Coban, T.; Kapucuoglu, O.; Raunio, H. The expression of cytochrome P450 enzymes in human breast tumours and normal breast tissue. *Breast Cancer Res. Treat.* **2001**, *70*, 47-54. doi: 10.1023/a:1012526406741.
35. Chen, F.; Li, Y.; Qin, N.; Wang, F.; Du, J.; Wang, C.; Du, F.; Jiang, T.; Jiang, Y.; Dai, J.; Hu, Z.; Shen, H. RNA-seq analysis identified hormone-related genes associated with prognosis of triple negative breast cancer. *J. Biomed. Res.* **2020**, *34*, 129-138.doi: 10.7555/JBR.34.20190111.
36. Aiyappa-Maudsley, R.; Storr, S.J.; Rakha E.A.; Green, A.R.; Ellis, I.O.; Martin, S.G. CYP2S1 and CYP2W1 expression is associated with patient survival in breast cancer. *J. Patho.l Clin. Res.* **2022**, *8*, 550-566.doi: 10.1002/cjp2.291.
37. Luo, B.; Chen, C.; Wu, X.; Yan, D.; Chen, F.; Yu, X.; Yuan, J. Cytochrome P450 2U1 Is a Novel Independent Prognostic Biomarker in Breast Cancer Patients. *Front Oncol.* **2020**, *10*, 1379. doi: 10.3389/fonc.2020.01379.
38. Justenhoven, C.; Winter S, Hamann U, Haas S, Fischer HP, Pesch B, Brüning T, Ko YD, Brauch H; GENICA Network.The frameshift polymorphism CYP3A43_74_delA is associated with poor differentiation of breast tumors. *Cancer* **2010**, *116*, 5358-64. doi: 10.1002/cncr.25508.
39. Stark, K.; Dostalek, M.; Guengerich, F.P. Expression and purification of orphan cytochrome P450 4X1 and oxidation of anandamide. *FEBS J.* **2008**, *275*, 3706–3717. doi: 10.1111/j.1742-4658.2008.06518.x.
40. Kim, J.; Kim, S.; Ko, S.; In, Y.-H.; Moon, H.-G.; Ahn, S.K.; Kim, M.K.; Lee, M.; Hwang, J.H.; Ju, Y.S.; Kim, J.I.; Noh, D.-Y.; Kim, S.; Park, J.-H.; Rhee, H.; Kim, S.; Han, W. Recurrent fusion transcripts detected by whole-transcriptome sequencing of 120 primary breast cancer samples. *Genes Chromosomes Cancer* **2015**, *54*, 681-91. doi: 10.1002/gcc.22279. Epub 2015 Jul 30.
41. Hlaváč, V.; Václavíková, R.; Brynychová, V.; Ostašov, P.; Koževnikovová, R.; Kopečková, K.; Vrána, D.; Gatěk, J.; Souček, P. Role of Genetic Variation in Cytochromes P450 in Breast Cancer Prognosis and Therapy Response. *Int. J. Mol. Sci.* **2021**, *22*, 2826. doi: 10.3390/ijms22062826.
42. Radvanyi, L.; Singh-Sandhu, D.; Gallichan, S.; Lovitt, C.; Pedyczak, A.; Mallo, G.; Gish, K.; Kwok, K.; Hanna, W.; Zubovits, J.; Armes, J.; Venter, D.; Hakimi, J.; Shorteed, J.; Donovan, M.; Parrington, M.; Dunn, P.; Oomen, R.; Tartaglia, J.; Berinstein, N.L. The gene associated with trichorhinophalangeal syndrome in humans is overexpressed in breast cancer. Proc. Natl. Acad. Sc.i U S A. 2005, 102, 11005-10. doi: 10.1073/pnas.0500904102.

 doi:10.20944/preprints202505.1835.v1

43. Cizkova, M.; Cizeron-Clairac, G.; Vacher, S; Susini, A.; Andrieu, C.; Lidereau, R.; Bièche, I. Gene expression profiling reveals new aspects of PIK3CA mutation in ERalpha-positive breast cancer: major implication of the Wnt signaling pathway. *PLoS One.* **2010**, *5*, e15647. doi: 10.1371/journal.pone.0015647.
44. Al-Saraireh, Y.M.; Alboaisa, N.S.; Alrawashdeh, H.M.; Hamdan, O.; Al-Saraireh, S.; Al-Shuneigat, J.M.; Nofal, M.N. Screening of cytochrome 4Z1 expression in human non-neoplastic, pre-neoplastic and neoplastic tissues. *Ecancermedicalscience* **2020**, *14*, 1114. doi: 10.3332/ecancer.2020.1114.
45. Liu, Z.; Gao, J.; Gu, R.; Shi, Y.; Hu, H.; Liu, J.; Huang, J.; Zhong, C.; Zhou, W.; Yang, Y.; Gong, C. Comprehensive Analysis of Transcriptomics and Genetic Alterations Identifies Potential Mechanisms Underlying Anthracycline Therapy Resistance in Breast Cancer. *Biomolecules* **2022**, *12*, 1834. doi: 10.3390/biom12121834.
46. Yang, X.; Hutter, M.; Goh, W.W.B.; Bureik, M. CYP4Z1 - A Human Cytochrome P450 Enzyme that Might Hold the Key to Curing Breast Cancer. *Curr. Pharm. Des.* **2017**, *23*, 2060-2064. doi: 10.2174/1381612823666170207150156.
47. Kozlov, A.P.Expression of evolutionarily novel genes in tumors. *Infect Agent Cancer*, **2016**, *11*, 34. doi: 10.1186/s13027-016-0077-6.
48. NCBI. CYP4Z1 - cytochrome P450 family 4 subfamily Z member 1, National Library of Medicine, National Center for Biotechnology Information, 2022. Available from: https://www.ncbi.nlm.nih.gov/gene/199974/ortholog/?scope=9347
49. Ortiz de Montellano, P.R. Mechanism and role of covalent heme binding in the CYP4 family of P450 enzymes and the mammalian peroxidases. *Drug Metab, Rev.* **2008**, *40*, 405-26. doi: 10.1080/03602530802186439.
50. Zöllner, A.; Dragan, C.A.; Pistorius, D.; Müller, R.; Bode, HB.; Peters, FT.; Maurer, H.H.; Bureik, M. Human CYP4Z1 catalyzes the in-chain hydroxylation of lauric acid and myristic acid. *Biol. Chem.* **2009**, *390*, 313-7. doi: 10.1515/BC.2009.030.
51. Yan, Q.; Machalz, D.; Zöllner, A.; Sorensen, E.J.; Wolber, G.; Bureik, M. Efficient substrate screening and inhibitor testing of human CYP4Z1 using permeabilized recombinant fission yeast. *Biochem. Pharmacol*. **2017**,*146*, 174-187.doi: 10.1016/j.bcp.2017.09.011.
52. McDonald, M.G.; Ray, S.; Amorosi, C.J.; Sitko, K.A.; Kowalski, J.P.; Paco, L.; Nath, A.; Gallis, B.; Totah, RA.; Dunham, M.J.; Fowler, D.M.; Rettie A.E. Expression and Functional Characterization of Breast Cancer-Associated Cytochrome P450 4Z1 in *Saccharomyces cerevisiae*. *Drug Metab. Dispos.* **2017**, *45*, 1364-1371. doi: 10.1124/dmd.117.078188.
53. Yuan, Y.; Yao, H.; Zhou, M.; Ma, X.; Zhou, Y.; Xu, J.; Niu, M.; Yin, J.; Zheng, L.; Xu, S. Identification of a Novel Potent CYP4Z1 Inhibitor Attenuating the Stemness of Breast Cancer Cells through Lead Optimization. *J. Med. Chem.* **2022**, *65*, 15749-15769. doi: 10.1021/acs.jmedchem.2c01320.
54. Guo, H.; Zeng, B.; Wang, L.; Ge, C.; Zuo, X.; Li, Y.; Ding, W.; Deng, L.; Zhang, J.; Qian, X.; Song, X.; Zhang, P. Knockdown CYP2S1 inhibits lung cancer cells proliferation and migration. *Cancer Biomark.* **2021**, 32, 531-539. doi: 10.3233/CBM-210189.
55. Fekry, M.I.; Xiao, Y.; Berg, J.Z.; Guengerich, F.P. A role for the orphan human cytochrome P450 2S1 in polyunsaturated fatty acid omega-1 hydroxylation using an untargeted metabolomic approach. *Drug Metab. Dispos.* **2019**, *47*, 1325-1332. doi: 10.1124/dmd.119.089086.
56. Beneke, A.; Guentsch, A.; Hillemann, A.; Zieseniss, A.; Swain, L.; Katschinski, D.M. Loss of PHD3 in myeloid cells dampens the in flammatory response and fibrosis after hind-limb ischemia. *Cell Death Dis.* **2017**, *8*, e2976. doi: 10.1038/cddis.2017.375.
57. Saarikoski, S.T.; Rivera, S.P.; Hankinson, O.; Husgafvel-Pursiainen, K. CYP2S1: A short review. *Toxicol. Appl. Pharmacol.* **2005**, *207*, 62–69. doi: 10.1016/j.taap.2004.12.027.
58. Bui, P.; Imaizumi, S.; Beedanagari, S.R.; Reddy, S.T.; Hankinson, O. Human CYP2S1 metabolizes cyclooxygenase- and lipoxygenase-derived eicosanoids. *Drug Metab. Dispos.* **2011**, *39*, 180–190. doi: 10.1124/dmd.110.035121.
59. Bui, P.H.; Hsu, E.L.; Hankinson, O. Fatty acid hydroperoxides support cytochrome P450 2S1-mediated bioactivation of ben zo[a]pyrene-7,8-dihydrodiol. *Mol. Pharmacol.* **2009**, *76*, 1044 1052. doi.org/10.1124/mol.109.057760.

 doi:10.20944/preprints202505.1835.v1

60. Nishida, C.R.; Lee, M.; de Montellano, P.R.O. Efficient hypoxic activation of the anticancer agent AQ4N by CYP2S1 and CYP2W1. *Mol. Pharmacol.* **2010**, *78*, 497-502. doi: 10.1124/mol.110.065045.
61. Mrízová, I.; Moserová, M.; Milichovský, J.; Šulc, M.; Kizek, R.; Kubáčková, K.; Arlt, V.M.; Stiborová, M. Heterologous expression of human cytochrome P450 2S1 in Escherichia coli and investiga tion of its role in metabolism of benzo[a]pyrene and ellipticine. *Monatsh. Chem.* **2016**, *147*, 881-888. doi.org/10.1007/s00706-016-1738-2.
62. Wang, K.; Guengerich FP. Bioactivation of fluorinated 2-aryl-benzothiazole antitumor molecules by human cytochrome P450s 1A1 and 2W1 and deactivation by cytochrome P450 2S1. *Chem. Res. Toxicol.* **2012**, *25*, 1740-51. doi: 10.1021/tx3001994.
63. Edler, D.; Stenstedt, K.; Öhrling, K.; Hallström, M.; Karlgren, M.; Ingelman-Sundberg, M.; Ragnhammar, P. The expression of the novel CYP2W1 enzyme is an independent prognostic factor in colorectal cancer-A pilot study. *Eur. J. Cancer* **2009**, 45, 705-712. doi: 10.1016/j.ejca.2008.11.031.
64. Esteller, M., Epigenetics in cancer. *N. Engl. J. Med.* **2008**, *358*, 1148–1159. doi: 10.1056/NEJMra072067.
65. Gomez, A.; Karlgren, M.; Edler, D.; Bernal, M.L.; Mkrtchian, S.; Ingelman-Sundberg, M. Expression of CYP2W1 in colon tumors: Regulation by gene methylation. *Pharmacogenomics* **2007**, *8*, 1315-1325. doi: 10.2217/14622416.8.10.1315.
66. Wang, Y.; Liu, Y.; Tang, T.; Luo, Y.; Stevens, M.F.G.; Cheng, X.; Yang, Y.; Shi, D.; Zhang, J.; Bradshaw, T.D. The antitumour activ ity of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole in human gastric cancer models is mediated by AhR signalling. *J. Cell. Mol. Med.* **2020**, *24*, 1750-1759. doi: 10.1111/jcmm.14869.
67. Ortiz de Montellano, P.R. Cytochrome P450-activated prodrugs. *Future Med. Chem.* **2013**, *5*, 213-28. doi: 10.4155/fmc.12.197.
68. Xiao, Y.; Guengerich, F.P. Metabolomic analysis and identification of a role for the orphan human cytochrome P450 2W1 in selective oxidation of lysophospholipids. *J. Lipid Res.* **2012**, *53*, 1610 1617. doi: 10.1194/jlr.M027185.
69. Zhao, Y.; Wan, D.; Yang, J.; Hammock, B.D.; Ortiz de Montella no, P.R. Catalytic activities of tumor-specific human cytochrome P450 CYP2W1 toward endogenous substrates. *Drug Metab. Dispos*. **2016**, *44*, 771-780. doi: 10.1124/dmd.116.069633.
70. Li, W.; Tang, Y.; Hoshino, T.; Neya, S. Molecular modeling of human cytochrome P450 2W1 and its interactions with substrates. *J. Mol. Graph. Model.* **2009**, *28*, 170-176. doi: 10.1016/j.jmgm.2009.06.002.
71. Guo, J.; Johansson, I.; Mkrtchian, S.; Ingelman-Sundberg, M. The CYP2W1 enzyme: regulation, properties and activation of pro drugs. *Drug Metab. Rev.* **2016**, *48*, 369-378. doi: 10.1080/03602532.2016.1188939.
72. Hodek, P.; Hrdinova, J.; Macova, I.; Soucek, P.; Mrizova, I.; Bur dova, K.; Kizek, R.; Hudecek, J.; Stiborova, M. Preparation and application of anti-peptide antibodies for detection of orphan cytochromes P450. *Neuroendocrinol. Lett.* **2015**, *36(Suppl. 1)*, 38-45. PMID: 26757124.
73. Dhers, L.; Ducassou, L.; Boucher, J.L.; Mansuy, D. Cytochrome P450 2U1, a very peculiar member of the human P450s family. *Cell. Mol. Life Sci.* **2017**, *74*, 1859-1869. doi: 10.1007/s00018-016-2443-3.
74. Guengerich, F.P. Human Cytochrome P450 Enzymes. In Cytochrome P450: Structure, Mechanism and Biochemistry, 3rd ed.; Ortiz de Montellano, P.R., Ed.; Kluwer Academic/Plenum Publishers: New York, NY, USA, 2005; pp. 377–530.
75. Nouri, K.; Pietrancosta, N.; Le Corre, L.; Dansette. P,M.; Mansuy, D.; Boucher, J-L. Human Orphan Cytochrome P450 2U1 Catalyzes the ω-Hydroxylation of Leukotriene B_4. *Int. J. Mol. Sci*. **2022**, *23*, 14615. doi: 10.3390/ijms232314615.
76. Kumar, S. Computational identification and binding analysis of orphan human cytochrome P450 4X1 enzyme with substrates. *BMC Res. Notes* **2015**, *8*, 9. doi: 10.1186/s13104-015-0976-4.
77. Zhang, Y.; Yuan, Z.; Shen, R.; Jiang, Y.; Xu, W.; Gu, M.; Gu, X. Identification of biomarkers predicting the chemotherapeutic outcomes of capecitabine and oxaliplatin in patients with gastric cancer. *Oncol. Lett.* **2020**, *20*, 290. doi: 10.3892/ol.2020.12153.

 doi:10.20944/preprints202505.1835.v1

disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.