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ORIGINAL ARTICLE

Preparation and application of anti-peptide antibodies for detection of orphan cytochromes P450

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Abstract

OBJECTIVES: Cytochromes P450 (CYP) are monooxygenases, which metabolize mostly hydrophobic endogenous and exogenous compounds. CYPs without any clear connection to metabolism are called "orphans". Interestingly, these "orphan" CYPs are over-expressed in tumor tissues. Thus, the main aim of the paper is the development of antibodies for immunodetection of these CYPs as potential malignancy markers.

METHODS: Unique sequences of CYP2S1 and 2W1 were selected and peptides synthesized. Chickens were immunized with peptides bound to hemocyanin (KLH). The antibodies were isolated from egg yolks and their reactivity was tested by ELISA. Antibodies were further affinity purified on immobilized peptides. Western blots containing CYP2S1 and 2W1 standards were developed with purified antibodies.

RESULTS: Using unique peptide immunogens of CYP2S1 and 2W1 the antibodies were developed. As judged from ELISA all chickens produced specific antibodies against the respective peptides. Both affinity purified antibodies against CYP2S1 peptide recognized the CYP2S1 standard on Western blots, but only one of four anti-peptide antibodies against CYP2W1 reacted with CYP2W1 standard. The antibodies were used for the detection of CYPs in cancer cell lines and human tissues samples. Although both CYPs were frequently co-expressed in cancer cells, CYP2S1 was solely induced in the cell line BxPC3, while CYP2W1 was predominantly present in cell lines MCF7 and HeLa. Our data show that anti-peptide antibodies are an indispensable tool for detection of homologous CYPs.

CONCLUSIONS: The anti-peptide antibodies successfully recognized CYP2S1 and 2W1 in the cancer cell lines and tissue samples.

Abbreviations

BCIP	- 5-bromo-4-chloro-3′-indolyphosphate
CNBr	- cyanogen bromide
CYP	- cytochrome P450
ELISA	- enzyme immunosorbent assay
IgY	- chicken yolk antibody
KLH	- keyhole limpet hemocyanin
NBT	- nitro-blue tetrazolium
PAGE	- polyacrylamide gel electrophoresis
PBS	- sodium phosphate buffered isotonic saline pH 7.4
PBS-Tw	- PBS containing 0.05% Tween 20
SD	- standard deviation
SDS	- sodium dodecyl sulfate
3D	- 3-dimensional

INTRODUCTION

Cytochromes P450 (CYP) are monooxygenases, which catalyze oxidative metabolism of endogenous (e.g. steroid hormones, prostaglandins, vitamin D, fatty acids) and exogenous compounds (e.g. drugs, carcinogens, pollutants). Of the 57 human CYP genes in 18 families, the members of the CYP1 to CYP4 families oxygenate mainly xenobiotics (and some endogenous compounds), whereas other CYP families are usually involved in highly specific metabolism of endogenous substrates (Nebert & Dalton 2006). There is a battery of CYPs without any clear connection to metabolism. Because of that these CYPs were originally called "orphans", CYPs lacking their specific substrates and functions. They are members of CYP2 and CYP4 families (Guengerich & Cheng 2011). Recently, great efforts have been made to "deorphanization" of these CYPs in respect of reactions they catalyze (Edson *et al.* 2013).

Interestingly, orphan CYPs belong among those which are also over-expressed in cancer cells. Immunochemical analyses indicated that orphan CYP2S1 is highly expressed in many human tumors of epithelial origin (Saarikoski *et al.* 2005). There is growing evidence that CYP2S1 participates in metabolism of retinoids during embryogenesis. Similarly to CYP1 family, the CYP2S1 is inducible by dioxin. In addition to involvement in metabolism of important endogenous substrates CYP2S1 was recently shown that metabolizes naphthalene (Karlgren *et al.* 2005). It is unclear why CYP2S1 is mostly expressed in tissues exposed to the environment, e.g., the gastrointestinal, respiratory, and urinary tracts. Likewise, another orphan CYP2W1 is expressed in several tumors, e.g. of colon and liver, but much less in corresponding normal cells. Moreover, the embryonic expression of CYP2W1 at protein and mRNA levels was confirmed in human fetal colon (Choong *et al.* 2015). The CYP2W1 expression in both colon and liver tumors negatively correlates with the prognosis of cancer patient survival. CYP2W1 can be induced by e.g. the antitumor agent imatinib and thus increased CYP2W1-mediated activation of prodrug (duocarmycin) and efficacy of therapy. However, the role of CYP2W1 in metabolism of endogenic compounds remains unknown. As tumorigenesis and embryogenesis are sharing common pathways the overexpression of CYP2S1 and CYP2W1 in tumors indicates the causal relation between their presence and a cancer progression. Hence, CYP2S1 and CYP2W1 have been suggested as markers of specific cancer development and their prognosis. In addition, these orphan CYPs are promising targets for an immunotherapy and the activation of prodrugs to cytotoxic metabolites in tumor tissues.

To develop an antibody diagnostic tool for detection of CYP2S1 and CYP2W1 expression in clinical tissue samples there are basically two approaches: the first is based on the CYP protein isolated from a biological sample or on the CYP protein recombinantly expressed and purified from cell lysate, the second makes the use of a synthetic peptide derived from the primary structure of the parent CYP protein. However, both ways of antibody preparation suffer from some drawbacks. The limitations of the "protein approach" arise from the difficulties with CYP expression as well as the protein purification. Moreover, because of CYP2S1 and CYP2W1 homology and more than 40% identity in their sequences, the antibody cross-reactivity is likely to occur between these CYPs and potentially among others of the CYP2 family. The "peptide approach" relies on the unique peptide sequence (at least 8 amino acid residues), the hapten, which is characteristic for the particular CYP only. To produce antibodies this peptidic hapten needs to be coupled to macromolecular carrier, usually protein, to prepare an immunogen. Nevertheless, the successful development of anti-peptide antibodies does not guarantee that they will recognize the peptide epitopes in the whole protein, which is on Western blots possibly denatured. Based on the literature data, the probability of generating a successful anti-protein antibody is up to 50%, depending on the peptide length immunogenicity and position in the protein structure (Hancock & O'Reilly 2005). Despite these limitations, anti-peptide antibodies can be extremely useful for protein detection when probing the expression of proteins e.g. on Western blots.

In the present study, we report the preparation of anti-peptide antibodies and their application as a diagnostic tool for detection of CYP2S1 and CYP2W1 expression in cancer cell line and tissue samples. Moreover, rabbit antibodies against CYP2S1 and CYP2W1 proteins were also tested for comparison.

MATERIAL AND METHODS

Chemicals

Bicinchoninic acid, anti-chicken IgG alkaline phosphatase conjugate, 5-bromo-4-chloro-3'-indolyphosphate/nitro-blue tetrazolium (BCIP/NBT) tablets CNBr-activated Sepharose, and Freund's adjuvant were purchased from Sigma Chemical Co. (St. Louis, MO); p-nitrophenyl phosphate and Tween 20 were from Serva (Heidelberg, Germany); Immobilon-P membrane from

Millipore (Bedford, MA); Pierce mcKLH and SulfoLink Coupling Resin from Thermo Scientific (Rockford, IL). All chemicals used in the experiments were of analytical grade purity or better. Antisera were prepared in rabbits immunized with recombinant CYP2S1 and CYP2W1 (Soucek 1999). Plasmid producing CYP2S1 and 2W1 were kindly provided by Prof. Guengerich (Vanderbilt Universtiy, Nashville, TN). Samples of cell lines and tumor tissues were kindly provided by Dr. Burdova (Institute of Molecular Genetics of the ASCR, CR) and Dr. Soucek (National Institute of Public Health, CR), respectively.

Peptide selection and immunogen preparation

Primary structures of human CYP2S1 (Entry #Q96SQ9) and CYP2W1 (Entry #Q8TAV3) were obtained from The UniProt Knowledgebase (http://www.uniprot.org). Their regular structures were calculated by Consensus Secondary Structure Prediction server of PBIL at Lyon-Gerland (https://npsa-prabi.ibcp.fr). Candidate peptide sequences were analyzed by Protein Analysis Tools on the ExPASy server (Gasteiger *et al.* 2005) and evaluated by on-line Invitrogen PeptideSelect service (http://rnaidesigner.lifetechnologies.com/peptide/design.do) and an AbDesigner tool (http://helixweb.nih.gov/AbDesigner/index.jsp). Three-dimensional structures of CYP2S1 and CYP2W1 were modeled by fully automated protein structure homology-modeling server at ExPASy (http://swissmodel.expasy.org) (Biasini *et al.* 2014). Peptide uniqueness was searched against the protein database by BLAST service at The National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). Selected peptides were synthesized by biotechnology company VIDIA (Jesenice, CR). To prepare immunogens, peptides were coupled to maleimide-activated keyhole limpet hemocyanin (KLH) *via* their Cys HS-groups according to manufacturer's manual (Thermo Scientific, Rockford, IL). In the case of peptide ending with Lys residue KLH was first modified with maleic anhydride to introduce HOOC-groups and then coupled *via* EDC with the peptide Lys H_2N-group (Butler *et al.* 1969).

Immunization and antibody preparation

Leghorn hens were immunized weekly by three subcutaneous injections with peptide-KLH conjugates (0.1 mg/dose/animal). The study was conducted in accordance with the Regulations for the Care and Use of Laboratory Animals (311/1997, Ministry of Agriculture, Czech Republic). Pre-immune IgY sample was purified from 6 eggs laid a week prior to immunization and the antigen-specific IgY sample from 6 eggs collected between 5–6 weeks after the immunization. IgY fractions were prepared from egg yolks by technique based on freezing-thawing of the water diluted yolks followed by a specific sodium chloride precipitation of IgY at low pH as described by Hodek *et al.* (2013). For purified IgY, the concentration ($mg.mL^{-1}$) was calculated from the absorbance at 280 nm using the experimentally determined factor of 1.094.

ELISA

The antibody immunoreactivity was tested by ELISA. An ELISA plate (Nunc-Maxisorp, Denmark) was coated with 100 µL per well of antigen solution (4 µg peptide. mL^{-1} in 50 $mmol.L^{-1}$ sodium carbonate buffer, pH 9.6) and incubated at 4 °C overnight. After washing 3 times with PBS containing 0.05% Tween 20 (PBS-Tw), each well was loaded with 150 µL of 2% solution of ovalbumin in PBS-Tw and incubated for 1 h at 37 °C. Wells were washed (3 times) with PBS-Tw and then in doublets loaded with 100 µL of antibody solution in PBS (pre-immune and immune IgY preparations, concentration series 3, 10 and 30 $\mu g.mL^{-1}$; affinity purified IgY fractions concentration series 0.3, 1.0, 3.0 and 10 µg. mL^{-1}). After washing (3 times) with PBS-Tw, 100 µL of alkaline phosphatase-conjugated rabbit anti-chicken IgG in PBS was added to each well (2000 times diluted commercial preparation, Sigma) and incubated at 37 °C for 1 hr. After washing with PBS-Tw, 100 µL of substrate solution (1 $mg.mL^{-1}$ p-nitrophenyl phosphate in carbonate buffer) was added and the plate kept for 10 min at room temperature. Reaction was stopped by 50 µL of 3 $mol.L^{-1}$ NaOH to each well and the color developed was measured at 405 nm with an ELISA reader ELX 800 (Bio-Tek Instruments, Winooski, VT).

Affinity purification of IgY fractions

To prepare affinity sorbent sulfhydryl-containing peptides were immobilized to SulfoLink coupling resin (1 mg of peptide per 1 mL of the gel) according to a reference manual. Solutions of anti-peptide CYP2S1 or CYP2W1 antibodies were end-over-end incubated with respective affinity sorbent at 4 °C overnight. The unbound IgY fraction was washed out with 1 $mol.L^{-1}$ NaCl in PBS. The specific anti-peptide antibodies were eluted with 50 $mmol.L^{-1}$ diethylamine (pH 11.5) and collected fractions (1 mL) were neutralized with 1 $mol.L^{-1}$ potassium phosphate (pH 6.7). Pooled fractions were dialyzed against PBS with 0.1% sodium azide and stabilized by the addition of bovine serum albumin (1 $mg.mL^{-1}$).

Western blot analysis

Western blotting was carried out as described earlier (Krizkova *et al.* 2008, 2009; Hodek *et al.* 2014; Kubickova *et al.* 2014). Protein concentrations in samples of recombinant CYP2S1 and CYP2W1, tissue lysates and sera were determined using the bicinchoninic acid protein assay with bovine serum albumin as the standard (Weichelman *et al.* 1988). The concentration of CYP was estimated according to Omura & Sato (1964) based on the absorption of the complex of reduced CYP with carbon monoxide. For sodium dodecyl sulfate (SDS)-electrophoresis (8% polyacrylamide gel), 15 µg protein/well of cell or tissue lysates were applied. The CYP2S1

and 2W1 proteins were detected by Western blotting on the Immobilon-P membrane (Millipore, Bedford, MA) using specific chicken anti-peptide CYP2S1 and 2W1 antibodies (15–30 μg.mL^{-1} or 1–5 μg.mL^{-1} for affinity purified IgYs). The visualization of protein bands on the membrane was performed using an anti-chicken IgG alkaline phosphatase-conjugated antibody (diluted 1:2,000) and BCIP/NBT substrate tablets (10 mg) for alkaline phosphatase.

RESULTS AND DISCUSSION

Orphan CYP2S1 and 2W1 are highly homologous and sharing more than 40% sequence identity. Thus to develop specific diagnostic antibodies for the detection of CYP2S1 and 2W1 in cancer cells the approach based on anti-peptide antibodies was chosen.

Peptide antigen design and immunogen construction

CYP2S1 and 2W1 primary structures were analyzed by free bioinformatics web services to design peptide antigens ensuring the production of the CYP specific antibodies. Unique peptides of CYP2S1 and 2W1 from sequence regions without any ordered structures having high scores of antigenicity, hydrophilicity and surface probability were selected from N- and C-terminus. The CYP2S1 and 2W1 peptide sequences for immunogen production are shown in Figure 1. In the case that there were no Cys or Lys residues present at any sequence terminus (peptides 2W1-B, 2W1-C or CYP2S1-B) Cys or Lys amino acid was added to provide the HS- or H_2N-group for an oriented coupling to carrier protein KLH. To prevent a random coupling of peptide CYP2S1-B to carrier protein KLH was first modified with maleic anhydride to introduce HOOC-groups for EDC-mediated condensation with the peptide Lys or N terminal H_2N-groups.

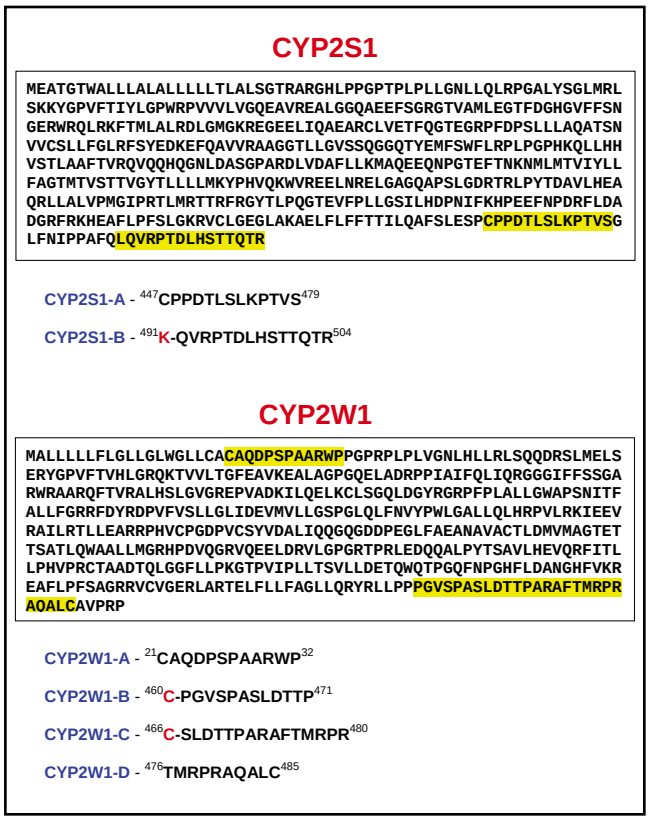

Fig. 1. Candidate sequences of CYP2S1 and 2W1 for the specific antibody production. In frames, there are the primary structures of both CYPs. The regions of the peptide selection are in yellow. An additional amino acid residue introduced is marked in red.

ELISA and affinity purification of anti-peptide IgYs

The antibodies were isolated from egg yolks using a water dilution & freezing/thawing technique followed by IgY specific precipitation with 1.5 mol.L^{-1} NaCl at pH 4. Of each chicken immunized with peptide immunogen two fractions, pre-immune IgY (control) and peptide-specific IgY, were prepared. When comparing total amounts of IgY protein per ml of yolks of these two fractions, the IgY production was almost doublet after chicken immunization (ranging 1.58–1.97 folds depending on the chicken). This elevation of IgY contents is in line with the hypersensitization of chickens with complete Freud's adjuvant as well as the formation of antibodies against the carrier protein KLH and also peptide. Then, the peptide specific immune responses of chickens were examined by ELISA. All IgY fractions prepared from egg yolks of immunized chickens were probed for the presence of anti-peptide IgYs using free peptides as coating antigens. The results of ELISA are present in Figure 2. Data indicate that the immunization of chickens with peptide immunogens proceeded successfully as all specific IgY fractions proved to react with corresponding peptide antigens.

Antibody fractions reacting with peptide antigens were further purified on affinity columns prepared by immobilization of peptides on a Sepharose gel. This purification step was carried out in order to prevent cross-reactivity of polyspecific IgYs present in a specific fraction with proteins of mammalian origin in cell or tissue lysates. Severe cross-reactivity of IgY fractions, which interfered with the CYP protein band detection on Western blots, has been described for e.g. human keratin (Macova *et al.* 2013). The progress of the purification of individual anti-peptide IgYs was monitored by IgY protein determination (based on absorbance at 280 nm) and by ELISA with the peptide antigen. Figure 3 shows a representative picture of the affinity chromatography used to prepare highly purified antibodies against two CYP2W1 peptides. To obtain anti-peptide IgY with high avidity towards the antigens loosely bound IgYs were eluted with 1 mol.L^{-1} NaCl prior to the elution of the main specific fraction. When comparing ELISA data for equally diluted IgY fractions before and after the affinity purification the anti-peptide IgY fractions were enriched 15–35 times (data not shown). The yields of affinity purified antibodies were 0.08–0.28% of total IgY amounts applied on the column.

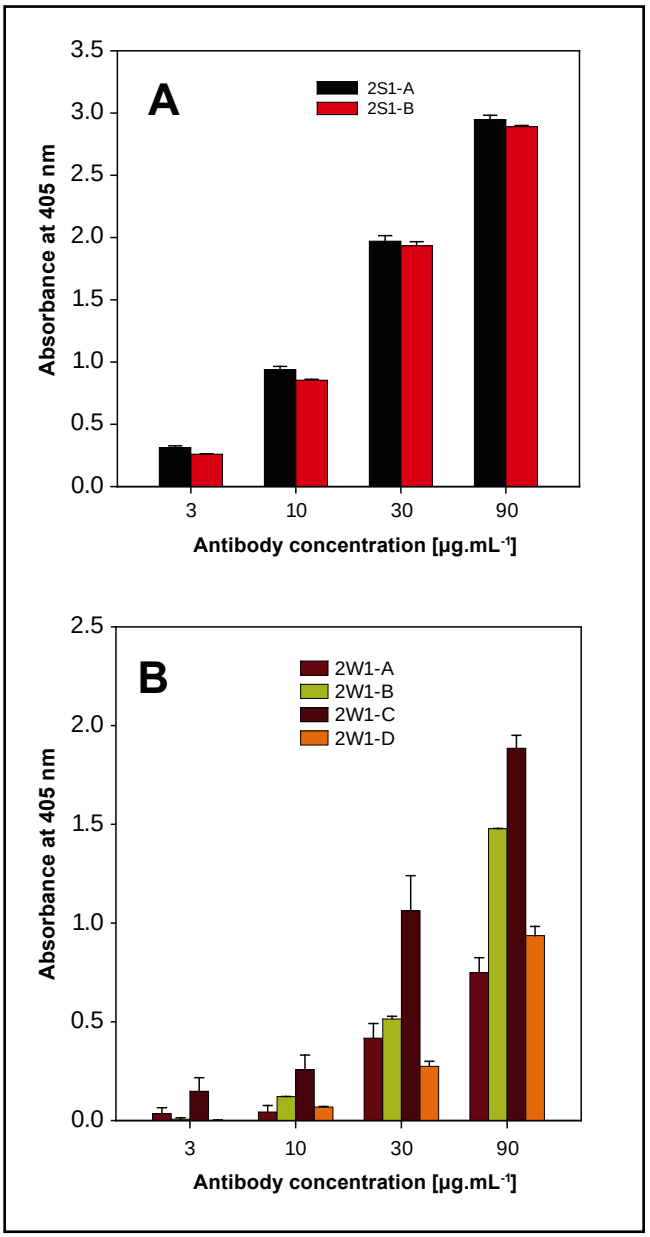

Fig. 2. ELISA of anti-peptide antibodies. As antigens peptides CYP2S1-A and CYP2S1-B or CYP2W1-A, CYP2W1-B, CYP2W1-C and CYP2W1-D were used for plates in panels **A** or **B**, respectively. Graph shows absorbance values at 405 nm of specific IgY fractions after subtraction of control values (pre-immune IgYs). The plotted data are means of triplicates ± SD.

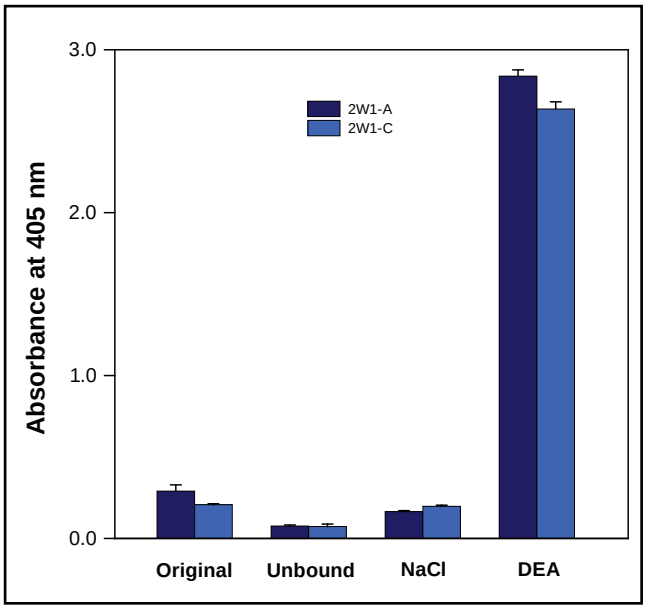

Fig. 3. Representative ELISA of IgY affinity chromatography. Anti-peptide antibodies were purified on immobilized peptides CYP2W1-A (dark blue bars) and CYP2W1-C (light blue bars). During the chromatography individual IgY fractions were collected: non-retained (UNBOUND), eluted with 1 mol.L^{-1} NaCl in PBS (NaCl), and eluted with 50 mmol.L^{-1} diethylamine, pH 11.5 (DEA). For comparison a non-purified IgY (ORIGINAL) was included. All IgY fractions were diluted to 10 µg.mL^{-1} of PBS. The plotted data are means of triplicates ± SD.

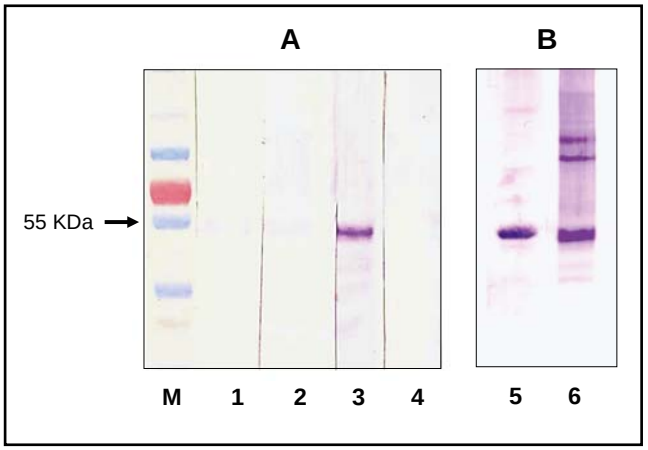

Fig. 4. Western blots of CYP2S1 and 2W1. Standards of CYP2W1 (panel **A**) and 2S1 (panel **B**) were electro-transferred on membranes and probed with affinity purified antibodies. Strips **1**, **2**, **3** and **4** containing CYP2W1 standard (2 pmol) were developed with antibodies (1 µg.mL^{-1}) against peptides CYP2W1-A, CYP2W1-B, CYP2W1-C and CYP2W1-D, respectively, while trips **5** and **6** containing CYP2S1 standard (1 pmol) were developed with antibodies (2 µg.mL^{-1}) against peptides CYP2W1-C and CYP2W1-D, respectively. In strip **M** the position of a protein standard with molecular weight 55 KDa is marked.

Western blots of CYP2S1 and 2W1

All six affinity purified anti-peptide IgYs were tested for their ability to react with the peptide epitope in CYP2S1 and 2W1 standards. Using Western blots, recombinantly expressed CYP standards were probed with anti-peptide IgYs. Both CYP2S1 anti-peptide antibodies reacted with a corresponding CYP standard, while only one of four CYP2W1 anti-peptide antibodies recognized a protein band of a CYP2W1 standard (see Figure 4). By their nature, anti-peptide antibodies are strictly site-directed probes for proteins. Both the sequence and position of the antibody epitope is quite important. Indeed, these antibodies are specific for a given linear peptide epitope occurring in the parent protein. Figure 5 shows the 3-dimensional (3D) homology model of CYP2W1 with highlighted peptides selected for the antibody production. Surprisingly, in the 3D CYP2W1 model the successful peptide epitope

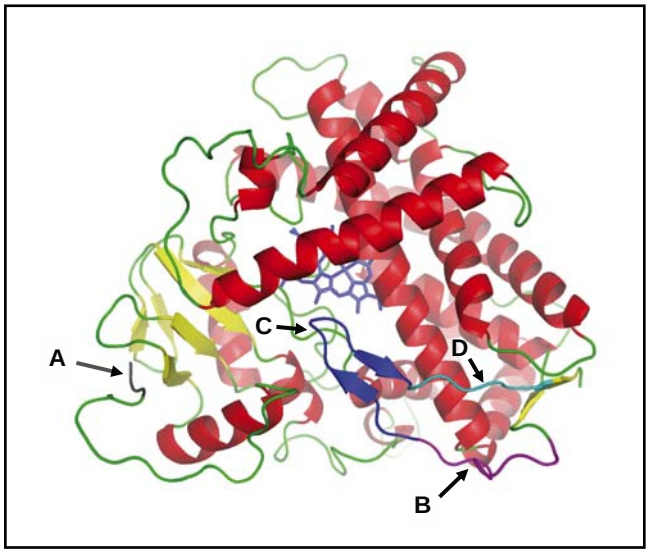

Fig. 5. Homology model of CYP2W1. Selected peptides CYP2W1-A (**A**), CYP2W1-B (**B**), CYP2W1-C (**C**) and CYP2W1-D (**D**) for the antibody production are highlighted in grey, magenta, blue and cyan, respectively.

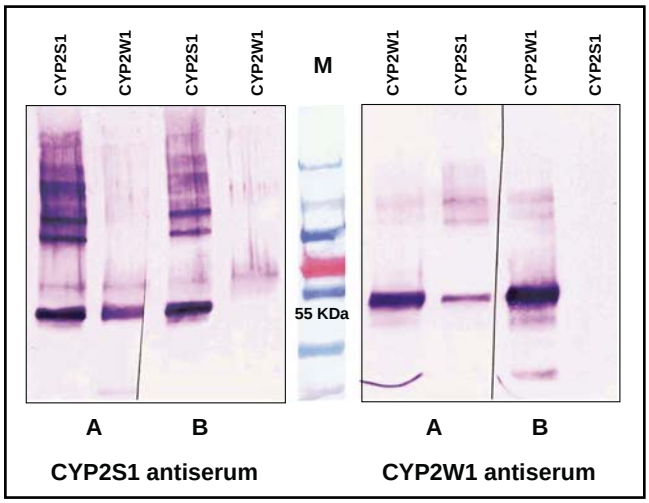

Fig. 6. Western blotting of CYP2W1 and CYP2S1 standards with rabbit antisera. Standards of CYP2S1 and 2W1 (1 pmol) were electro-transferred from PAGE to membranes and probed with corresponding rabbit antisera (5 μg.mL^{-1}) prior (**A**) and after (**B**) their purification on cross-reacting proteins. In strip **M** the position of a protein standard with molecular weight 55 KDa is marked in blue (bellow the red band).

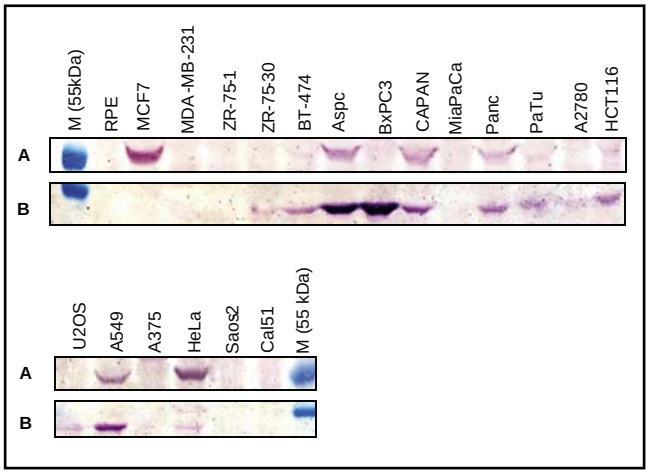

Fig. 7. Detection of CYP2W1 and CYP2S1 in biological samples. Western blotting was carried out with cell lysates (30 μg protein per well), which were electro-transferred from PAGE to membranes and probed with affinity purified anti-peptide IgYs against human CYP2W1 and CYP2S1. The abbreviations of the cell samples are explained in Table 1. The line **A** and **B** were developed with anti-peptide antibodies (5 μg.mL^{-1}) against human CYP2W1 and CYP2S1, respectively. The position of a protein standard with molecular weight 55 KDa (**M**) is shown in blue.

CYP2W1-C is forming a hairpin loop containing two short antiparallel β-sheets. Likewise, all peptide scores for proceeding CYP2W1-B or following CYP2W1-D peptides were better than those for CYP2W1-C. This precise evaluation of the CYP2W1 sequence part (460–485) clearly documents that *in silico* predictions are of a limited use only. Taking together the results of our antigen predictions have met the 50% probability of successful matches (three functional peptide antigens of six selected) that is in accordance with published data (Hancock & O'Reilly 2005). Finally, it should be noted that CYP2S1 and 2W1 anti-peptide antibodies did not show any cross-reactivity with CYP2W1 and 2S1 standards.

To confirm our results obtained with anti-peptide IgYs identical blots were also developed with rabbit antisera raised against CYP2S1 and 2W1 proteins expressed in *E. coli*. The results shown in Figure 6, however, depict a high cross-reactivity of both antisera with CYP2S1 and 2W1 standards. Furthermore, an antiserum against e.g. CYP2W1 reacted also with a human CYP2B6 standard (data not shown). This cross-reactivity problem of CYP2S1 antiserum was circumvented by trapping of the IgG fraction recognizing CYP2W1 with immobilized CYP2W1 on gel beads and *vise versa* with CYP2W1 antiserum. Although rather high amounts of IgG (47% and 63% for CYP2S1 and 2W1 antisera, respectively) were removed from the original samples, this procedure was necessary to improve the antisera specifity. Blots presented in Figure 6 confirm that the cross-reactivity of rabbit antisera was entirely eliminated by their treatment with crossing antigens. The developed protein bands with higher molecular weights than that of a parent protein are most probable bands of di- and trimers of CYP2S1.

Immunodetection of CYP2S1 and 2W1 in biological samples

Immunoaffinity enriched anti-peptide antibodies were employed for the detection of CYP2S1 and 2W1 expression in cancer cell lines and tissue samples. The results of a Western blot analysis of tumour whole cell lysates are collectively shown in Figure 7. The presence of CYP2W1 was detected in MCF7, Aspc, CAPAN,

Panc, A549 and HeLa cell lysates and in a small amouns in BT-474, PaTu and HCT116 cell lysates (for cell line description see Table 1). A band of unknown identity was observed at approximately 62 kDa at the BxPC3 cell line (not shown). This band may belong to the glycosylated form of the CYP2W1 (Gomez *et al.* 2010). The expression of CYP2S1 protein was determined in Aspc, BxPC3, CAPAN and A549 whole cell lysates. In addition low levels of CYP2S1 expression was observed in ZR-75-30, BT-474, Panc, PaTu, A2780, HCT116, U2OS and HeLa cell lysates, too. It is interesting to note, that the presence of both studied proteins, CYP2W1 and CYP2S1, was determined in the majority of pancreatic tumour (except of MiaPaCa), cervical cancer (HeLa), colorectal cancer (HCT116) and a lung adenocarcinoma (A549) cell lines. On the other hand, the presence of CYP2W1 was determined just in one out of six studied breast cancer cell lines (MCF7) and in the case of CYP2S1 in only two out of six studied breast cancer cell lines (ZR-75-30, BT-474). These findings are consistent with our previous findings, showing CYP2W1 not to be a proper independent biomarker for breast carcinoma, because its expression in breast tumors is not high enough (Hlavac *et al.* 2014). The CYP2W1 may rather be used as diagnostics tool for patients with stages II and III of colorectal cancer (Edler *et al.* 2009), while CYP2S1 may serve as a prognostic marker in both, colorectal and breast cancers (Kumarakulasingham *et al.* 2005; Murray *et al.* 2010). Since the elevated expression of CYP2W1 in pancreatic tumor cells was determined by our anti-peptide antibody as well as by immunohistochemical staining (Uhlen *et al.* 2010) this CYP should be also considered as a specific for pancreatic cancer. Likewise, we found increased levels of CYP2S1 in pancreatic tumor cells (in five out of six cell lines). However this result is inconsistent with immunohistochemical staining of eleven studied pancreas tumor tissue (Uhlen *et al.* 2010).

The present data indicate that the anti-peptide antibodies were successfully employed for the screening of the individual expression of CYP2S1 and 2W1, highly homologous CYPs, in a battery of cell lines and tissue samples. These chicken yolk antibodies may serve as markers of various malignancies.

Tab. 1. Human cells used for CYP2S1 and CYP2W1 detection.

Biological material	Specification
RPE	transformed retinal pigment epithelial cell line
U2OS	bone osteosarcoma epithelial cell line
A549	lung adenocarcinoma epithelial cell line
A375	malignant melanoma cell line
HeLa	cervical cancer cell line
Saos-2	osteosarcoma cell line
Cal51	breast cancer cell lines
MCF7	
MDA-MB-231	
ZR-75-1	
ZR-75-30	
BT-474	
Aspc	pancreatic cancer cell lines
BxPC3	
CAPAN	
MiaPaCa	
Panc	
PaTu	
A2780	ovarian carcinoma cell line
HCT116	colorectal carcinoma cell lines

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