doi: 10.4149/gpb_2020043

CYP3A gene variability and cancer cells response to the treatment

Martina Šemeláková[1], Lenka Maliničová[2], Mária Macejová[2] and Peter Pristaš[2]

[1] *Department of Medical Biology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovakia*
[2] *Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Košice, Slovakia*

Abstract. The treatment of cancer depends on the activity of the cytochrome P450 enzyme family, which is essentially carried out by the CYP3A4 and CYP3A5 enzymes. The aim of our study was to investigate whether the CYP3A4 polymorphism could contribute to protein activity and their influence to the response of cancer cells to treatment. The variability of *CYP3A4* cDNA profiles between the cancer cell lines parental HT-29 and resistant HT-29-OxR adenocarcinoma was detected using denaturing gradient gel electrophoresis (DGGE). Subsequently, sequence analysis of *CYP3A* family members (*CYP3A4, CYP3A5*) confirmed polymorphism of the *CYP3A4* gene in studied cancer cell lines. Variations at the gene expression level, the protein level and the activity of CYP3A4 protein in 12 cancer cell lines were observed, also different response to drug treatments between cell line HT-29 and oxaliplatin-resistant cell line HT-29-OxR. The variability of *CYP3A* might affect the efficiency of anti-cancer drugs in general and have an impact on metabolism.

Key words: Polymorphism — Cancer — CYP3A4 — CYP3A5 — HT-29 — Resistance — DGGE

Abbreviations: ATCC, American Tissue Culture Collection; CYP3A4, cytochrome P450 3A4 enzyme; DGGE, denaturing gradient gel electrophoresis; HP, hyperforin; HT-29, human colon adenocarcinoma cell line; HT-29-OxR, human colon adenocarcinoma cell line oxaliplatin resistant; HYP, hypericin; RPMI-1640, cell growth medium.

Introduction

The most accepted argument for resistance to anti-cancer drugs is the expression of transporters that identify and banish anti-cancer drugs from cells and the activation of drug-detoxifying system (Gottesman 2002; Fletcher et al. 2010; Cascorbi 2012; Sun et al. 2012; Avril et al. 2017). The human CYP superfamily consists of a family, subfamilies, and individual members consisting of 57 functional genes, the most important group of phase I drug metabolizing enzymes, and 58 pseudogenes cooperate on inactivation of carcinogens and anticancer drugs (Huttunen et al. 2008), with important roles in cancer diagnosis and as determinants of treatment. Therefore the diverse P450 (CYP) enzyme superfamily is the most important complex involved in the biotransformation of many drugs, toxins or cancer-causing agents (Oyama et al. 2004). Drug metabolism steps are divided into funcional state, conjunction and transport of proteins, significantly carried out by CYP3A4 (Xu et al. 2005). The effect of variations in the DNA sequence of specific genes affect the drug response studies of pharmacogenetics. Polymorphisms in the CYP family can have largest impact on the designation of therapeutic drugs. *CYP2D6, 2C19* and *2C9* polymorphisms were reported for highest frequent variations in metabolism of drugs. Large polymorphism also appear in the *CYP1A1*, *2A6*, *2A13*, *2C8*, *3A4* and *3A5* genes, which may contribute to the variable sensibility to carcinogenesis. The spreading of the frequent variant alleles of *CYP* genes also differs between various ethnic populations (Zhou et al. 2009). Many variation analyses of genes associated with cancer have been carrying out (Pharoah et al. 2007).

Denaturing gradient gel electrophoresis (DGGE) analysis is used for separation based on the lower electrophoretic mobility of a partially melted double-stranded DNA molecule, but also, well-separated bands can be extracted

Electronic supplementary material. The online version of this article (doi: 10.4149/gpb_2020043) contains Supplementary Material.

Correspondence to: Martina Šemeláková, Department of Medical Biology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Trieda SNP 1, 040 11 Košice, Slovakia
E-mail: martina.semelakova@upjs.sk

from the gel to be sequenced and identified. Previously was used DGGE method, based on differences in GC content for describing the MSH2 mutation of the *HNPCC* gene (Wijnen et al. 1994), *BRCA1* exons (Hoya et al. 1999), *TP53* gene mutations in human lung cancer (Holmila et al. 2006), oncogene mutations (Guldberg et al. 2002) and phospholipase D in non-small cell lung cancer (NSCLC) (Ahn et al. 2012). At first here was DGGE analysis used for distinguishing *CYP3A4* mRNA alternative transcripts in different cancer cell lines, because we assumed that different cancer cell lines could contain CYP3A4 transcript variants. Otherwise, all isolates from different cancer cell lines produced multiband profiles, though the band number does not correlate directly with the transcript variants. The sequences from separated *CYP3A4*-DGGE fragments from cell line HT-29-OxR were compared to the available transcript variants of the *CYP3A4* gene from GenBank. Moreover, CYP3A4 protein level was evaluated between cancer cell lines using Western blot, followed by luminometric analysis of protein activity. Our study contains selected cancer cell-line models of Caucasians, because distribution of the common variant alleles of the *CYP* genes varies among ethnic populations.

Therefore, as described previously (Semelakova et al. 2012), we focused on the study of photodynamic therapy processes after treatment for various cancer diseases, using two bioactive drugs isolated from *Hypericum* sp. (St. John's wort): hypericin (HYP), which demonstrated photocytotoxic activity *in vitro* and *in vivo* (Agostinis et al. 2000), and the study of hyperforin (HP) (Erdelmeier 1998). Based the results, we decided that transcriptional variants of *CYP3A4* may have an impact on the efficiency of anti-cancer drugs through their effects on the activity of CYP3A4 proteins. A new perspective on approaches to the analysis of transcripts of different genes and their impact on the activity of proteins we recommend through the study of transcription variants.

Materials and Methods

Cell cultures

Human cell lines were cultured in RPMI-1640 supplemented with 10% fetal calf serum and antibiotics (streptomycin 100 μg/ml, penicillin 100 U/ml, Invitrogen Co., Carlsbad, CA, USA). Cells were cultivated at 37°C in a humidified 5% CO_2 atmosphere. The HUVEC cell line (human umbilical vein endothelial cells, from umbilical vein, isolated from newborn, adherent cell culture) was kindly provided by Department of Pharmacology (P. J. Šafárik University) (Varinska et al. 2018); the human colon adenocarcinoma cell line HT-29 (human caucasian colon adenocarcinoma, adherent cells, isolated from a primary tumour in 44 year old caucasian female) was purchased from American Tissue Culture Collection (ATCC, Rockville, MD, USA); HT-29-OxR (oxaliplatin resistant) kindly provided by Institute of Biophysics, Brno, Czech Republic (Strakova et al. 2013), an *in vitro* model of the CRC chemoresistance cell line was prepared by chronic exposure of human CRCs (HT-29) to increasing doses of oxaliplatin. Oxaliplatin is the first compound in the colorectal carcinoma treatment and resistance to oxaliplatin is multifactorial, and includes low-efficient cellular drug uptake and accumulation. The following cell lines were used: human ovarian carcinoma A2780 (ATCC, Rockville, MD, USA); CDDP-resistant subline A2780cis, cytostatic-drug resistant, cisplatine resistant A2780 ovarian cancer cell line (Kozubík et al. 2008) kindly provided by Institute of Biophysics, Brno, Czech Republic (Sigma 93112517); HL60 human caucasian acute promyelocytic leukemia (American Tissue Culture Collection (ATCC), Rockville, MD, USA); HL60+MRP1 (ABCC1-overexpressing subclone) (Szatmari et al. 2006), HL60+MRP1 human promyelocytic leukemia cells overexpressing ABC transporters related to MDR (HL60-MDR1, HL60-MRP1, and PLB-BCRP) kindly provided by prof. Sarkadi (Membrane Research Group, Hungarian Academy of Sciences, Budapest, Hungary). The cell lines lung epithelial adenocarcinoma A549 (adenocarcinomic caucasian human alveolar basal epithelial cells) (ATCC, CCL-185), acute myeloid leukemia UT7 (established from the bone marrow of a 64-year-old man with acute myeloid leukemia AML M7, ACC137), colorectal carcinoma HCT116 (human colon carcinoma, malignant cells isolated from a male, ATCC, CCL-247™), renal adenocarcinoma 769P (caucasian female adherent cells, ATCC® CRL-1933™), breast adenocarcinoma MCF7 (epitelial mammary gland, breast, caucasian female, ATCC, HTB-22) and mammary gland carcinoma T47D (adherent female ductal carcinoma, ATCC HTB-133), Jurkat cells (human blood, leukemic T-cell lymphoblast (ATCC® TIB-152™), cervical cancer HeLa cells (ATCC® CCL-2) were purchased from ATCC (Rockville, MD, USA).

RNA and DNA isolation, reverse transcription and PCR analysis

Total RNA was isolated from the specified cell lines using TRIzol® reagent (Invitrogen) according to the manufacturer's instructions. Total RNA concentration and purity (OD 260/280 ratio and OD 260/230 ratio) were spectrophotometrically evaluated (BioSpec-nano Shimadzu) (Life Science). Reverse transcription (RT) was performed at 37°C in a 20 μl volume using 1 μg of the total RNA, 10 mM random hexamer primer and 200U M-MLV reverse transcriptase (Invitrogen) according to the manufacturer's instructions. The most variable sequence of the complete

CYP3A4 gene (AF182273) in the range from 661 up to 960 nucleotides (Chromosome 7/ map="7q22.1") was selected for analysis. The PCR for *CYP3A4* with primers CYP4-For: 5'-TCAGCCTGGTGCTCCTCTAT-3', and CYP4MS-Rev: 5'-AGGAATGGAAAGACTGTTATTGAGA-3'with GC clamp was performed in the C1000 Touch thermal cycler (BioRad, Hercules, CA, USA). The analysis of *CYP3A5*1* and **3* and *CYP3A4*11* was performed from DNA templates, with primers *CYP3A4*11* (Lee et al. 2005), with primers for the *CYP3A5* gene CYP5MS-For: 5'-CATCTTTGGGGCCTACAGCA-3', CYP5MS-Rev: CCACCATTGACCCTTTGGGA, primers for *CYP3A5*1* and *CYP3A5*3* (Westlind-Johnsson et al. 2003) in 25 µl reaction volume containing: 1× PCR Master Mix (Thermo Fisher Scientific), 0.5 mM forward and reverse primer and 50 ng of reverse transcribed RNA/cDNA. The PCR reaction conditions were as follows: 7 min at 95°C, 40 cycles of 30 s at 94°C, 30 s at 57°C (for *CYP3A4)* or 61°C (for *CYP3A5)*, 30 s at 72°C and 5 min at 72°C. The successful amplification of cDNA was confirmed by electrophoresis on 0.8% agarose gel in TAE buffer (Thermo Fisher Scientific) and visualised with 0.5 µg/ml GelRed™ (Biotium Inc.).

qRT-PCR analysis

Quantitative real-time PCR analysis for CYP3A4 and ß-actin was performed in triplicate using an iCycler iQ Real-Time PCR Detection System (BioRad, USA) in a 25 ml reaction volume containing: 1 × iQTM Sybr1Green Supermix (Bio-Rad), 0.5 mM forward and reverse primers and 50 ng of reverse transcribed RNA/cDNA. The reaction conditions were as follows: 7 min at 95°C, 40 cycles of 30 s at 94°C, 30 s at 57°C (*ß-actin*, *CYP3A4*), 30 s at 72°C and 5 min at 72°C followed by melting curve analysis to confirm amplification of the specific product. Relative quantification was used with the comparative CT method, and data were normalised to the housekeeping gene *ß-actin*.

Denaturing gradient gel electrophoresis (DGGE)

DGGE was performed on the DCodeTM Universal Mutation Detection system (16 cm system; Bio-Rad, Hempstead, UK). DGGE parallel gradient gel ranged from 30 to 40% (6% 37.5:1 40%(w/v)acrylamide), run at 50 V, 200 mA, 250 W for 20 h at 60°C. DNA was visualized by staining with SYBR Gold nucleic acid gel stain (Molecular Probes, Eugene OR, USA). The PCR products of *CYP3A4* mRNA transcripts of the tested cell lines were compared.

DNA sequencing and sequence analyses

The fragments A to D were extracted from the DGGE gel and reamplified using the same primer pair. Other tested fragments were identified by reciprocal PCR analysis as artefacts and thus excluded from analysis. The PCR products were purified using the GeneJET PCR Purification Kit (Thermo Fisher Scientific), ligated into the vector pTZ57R/T and cloned into *Escherichia coli* (strain HB101), following the protocol of the Thermoscientific InsTA Clone PCR Cloning Kit (Thermo Fisher Scientific). Plasmids were isolated from recombinant colonies and checked for inserts. Those with inserts were sequenced in both directions on a Beckmann DNA Sequencer, and the inserts were verified as being *CYP3A4* genes by DNA sequence analysis using BLASTN.

PCR amplicons were purified and sequenced using the same primers as for PCR amplification with the ABI 3730 capillary sequencer. The available sequences of *CYP3A4*, *CYP3A5*, *CYP3A43* and *CYP3A7* from the CYP3A family in GenBank were aligned using the ClustalW algorithm implemented in MEGA 6 software (Tamura 2013), and relationship trees were constructed using the Maximum Likelihood (ML) reconstruction method of analysis available in this software.

Western blot analysis

For Western blot analysis, cells were collected and lysed in lysis buffer (100 mM Tris-HCl, pH 7.4; 1% SDS, 10% glycerol) supplemented with a protease inhibitor cocktail (P2714, Sigma-Aldrich) for 10 min on ice. The cell lysates were sonicated for 30 s at 30% power of the Bandelin Sonopuls HD2070 (Bandelin electronic, Berlin, Germany) on ice and centrifuged for 10 min/13.000 × *g* at 4°C. Protein concentration was determined using a detergent-compatible protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Equal protein amounts (30 µg) supplemented with 0.01% bromphenol blue, 1% 2-mercaptoethanol, 0.4% SDS and 5% glycerol were then separated with 10% SDS-polyacrylamide gel (Acrylamide/Bis Solution, 37.5:1) and transferred (wet transfer, 80 min) onto a PVDF membrane (Bio-Rad Laboratories) in a transfer buffer (192 mM glycine, 25 mM Tris and 10% methanol). After 1 h of membrane blocking at RT in 5% non-fat milk (20 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% TWEEN 20, pH 7.4), the PVDF membrane blots were incubated overnight at 4°C with primary CYP3A4 antibody (mouse monoclonal HL3: sc-53850). After 20 min washing in wash buffer, the membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibody for 1 h at RT (anti-mouse IgG- HRP, sc-516102, 1:5000, Santa Cruz). Detection of antibody reactivity was performed using a Pierce ECL Western Blotting Substrate (Pierce) and visualized on X-ray films (Agfa Healthcare NV, Belgium). Equal sample loading was verified by immunodetection of β-actin (A5441, 1:6000, Sigma-Aldrich). The densitometry of the

CYP3A4 proteins was evaluated using ImageJ software and normalized to β-actin protein levels.

Luminometric analysis of CYP3A4 activity

Finally, the aim was to investigate whether the CYP3A4 polymorphism contributes to the variability in CYP3A activity and the response to treatment of cancer cells. The analysis of protein CYP3A4 activity was performed on two cell lines, HT-29 and resistant HT-29-OxR. CYP3A4 metabolic activity was evaluated in cells after drug treatment on the HT-29 and HT-29-OxR cell lines using the Promega CYP3A4/Luciferin-IPA P450-Glo™ Assay (Promega Cat No. V9001) with Luciferin-IPA, a luminogenic CYP3A4-specific substrate.

The treatment protocol followed the principles of photodynamic therapy (PDT), concentrations and types of drugs and doses of light (Semelakova et al. 2012). Nonlytic Assays were performed according to protocol in a 96-well plate format. The 20×10^3 cells/well in the 96-well plate were incubated for 24 h in the dark, then treated with 100 nM HYP (CAS No. 548-04-9; HPLC grade, AppliChem GmbH, Germany) and then 20 μM HP (CAS 110 79-53-1; AppliChem, Germany) for the next 20 h. The groups treated by PDT followed the procedure of cell photosensitization on the diffuser glass of the irradiating device in sequence and were exposed to irradiation for specific time periods to reach 3.15 J/cm^2. Our irradiation device consisted of L18W/30 (Osram, Berlin, Germany) fluorescent tubes with maximum emission in the range of λ = 530–620 nm, which covers HYP maximum absorbance (590–600 nm) (see Supplementary Material, Fig. S1). The irradiator fluence rate was measured at the diffuser surface. The experiment was performed following the protocol of CYP3A4/Luciferin-IPA P450-Glo™ Assays, and the emitted luminescence was finally measured using a luminometer FLUOStar Optima (BMG Labtechnologies GmbH, Germany) according to the experimental scheme. The activity of CYP3A4 was evaluated as the luminiscence ratio of each experimental group to the untreated group. The luminiscence content was quantified as the ratio of experimental groups treated with HYP or HP alone to the untreated control.

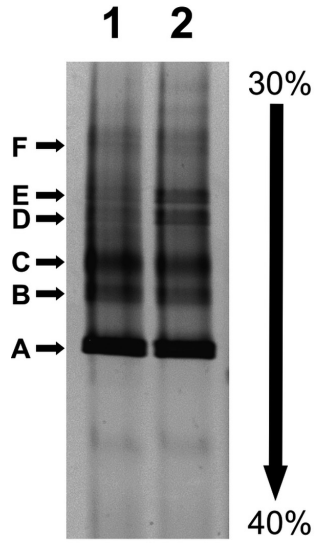

Figure 1. Denaturing gradient gel electrophoresis (DGGE) analyses of the gene products CYP3A4. DGGE parallel gradient gel ranged from 30 to 40% (6% 37.5:1 40% (w/v)) acrylamide. The PCR products of *CYP3A4* mRNA transcripts of the tested cell lines: HT-29, HT-29-OxR. The fragments A to E were isolated and sequenced.

Statistical analysis

Data were processed as means ± SD from three independent experiments. Results were analyzed using one-way ANOVA with Tukey's *post-hoc* test at three statistical levels: $p < 0.001$, $p < 0.01$ and $p < 0.05$. Groups treated with HYP or HP were compared with the control and the experimental groups.

Results

The gene products CYP3A4 analysed by DGGE

The PCR products of transcripts of *CYP3A4* mRNA were compared in different cell lines using DGGE. Cell lines HT-29 and HT-29-OxR showed a profiles, differed in the intensity of individual fragments (Fig. 1). Set of fragments was detected in all tested cell lines (HT-29, HT-29-OxR, Acis, A2780, HL60, HL60+MRP1, A549, UT7) (data not shown). Similarly, ovarian carcinoma A2780 and ovarian carcinoma resistant line Acis exhibited a similar number of bands, differed by the intensity of one of fragments. Lung adenocarcinoma A549 and acute myeloid leukemia UT7 also showed a similar fragments profile. The DGGE analysis was able to distinguish the profiles of all sets of CYP3A4 products in individual cell lines. Every cell line presented the fragments A of CYP3A4, cell line HL60 expressed the same concentration of fragment A as HT-29 and its resistant form HT-29-OxR. Moreover, the cell line A549 and UT7 proved the fragment C of CYP3A4 as HT-29 and HT-29-OxR. The DGGE profiles of the CYP3A4 gene in the other cell lines compared were different.

Sequence and Maximum Likelihood analysis

The sequence analysis was used to assign function to genes and proteins by the study of the similarities between the compared sequences. Selected *CYP3A4* and *CYP3A5* PCR

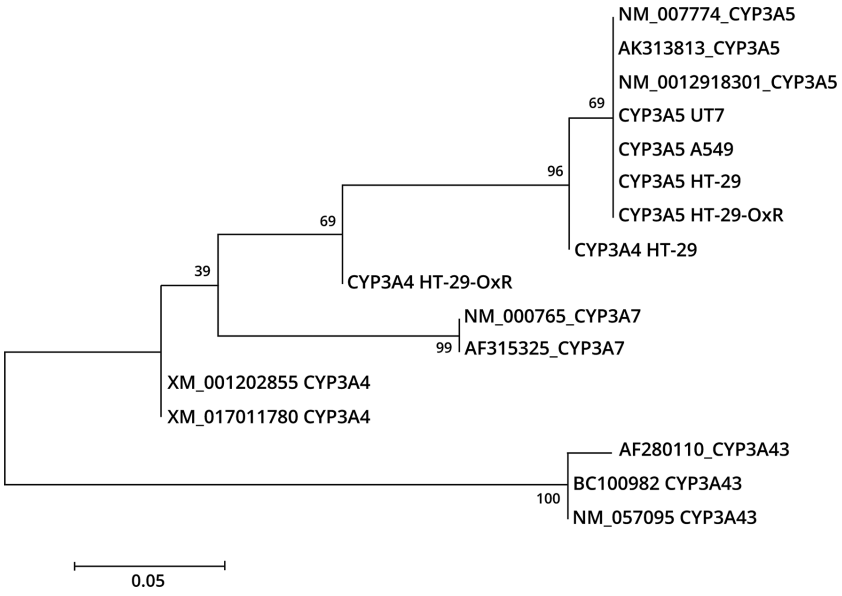

Figure 2. Sequence analysis (Maximum Likelihood (ML) analysis) of the relationship between genes for enzymes of the CYP3A group of *CYP3A4, CYP3A5, CYP3A7, CYP3A43* isoenzymes were aligned using the available sequences from the CYP3A family in GenBank, constructed by the ClustalW algorithm in MEGA6 software.

amplicons were cloned, completely sequenced and aligned, and the evolutionary distances were computed using the Maximum Composite Likelihood method in the unit of number of base substitutions *per* site (Fig. 2).

The sequence comparisons confirmed polymorphism of the *CYP3A4* but showed any variability of *CYP3A5* gene. While partial HT-29 *CYP3A4* aminoacid sequence is identical to the wild type of human *CYP3A4*, oxaliplatin resistant variant HT-29-OxR differs by single mutation, lung adenocarcinoma A549 differs by multiple mutation in analysed *CYP3A4* region. Polymorphism of the *CYP3A4* gene led to the production of a slightly modify protein with several mutations (Fig. 3) influenced CYP3A4 activity.

Western blot determination of the CYP3A4 protein level

A different level of CYP3A4 protein [51 kDa] was detected in presented cell lines HT-29, HT-29-OxR, HCT116, A549, UT7, 769P, and T47D using Western blot analysis (Fig. 4). The three groups of protein level were distinguished based on the level of protein by comparing the levels of all cell lines with each other. The first group, with a low level of protein was detected in cell lines A2780cis, myeloid leukemia UT7, lung adenocarcinoma A549, renal adenocarcinoma 769-P, HL60 and Jurkat. The second group, with a medium level of protein, included the cell lines HCT116, A2780, breast adenocarcinoma MCF7, mammary gland carcinoma T47D and HeLa cells. The third group, with a high level of CYP3A4 protein, included HUVEC, colorectal adenocarcinoma HT-29, HT-29-OxR and acute promyelocytic leukemia HL60+MRP1 (ABCC1-overexpressing subclone). Western blot method showed the content of CYP3A4 protein, while OD show the protein level CYP3A4 to level of β-actin calculated by ImageJ analysis (CYP3A4/β actin).

qRT-PCR analysis of CYP3A4

Different levels of *CYP3A4* gene expression were demonstrated in tested cancer cell lines by qRT-PCR analysis (Fig. 5), while the analysis monitored the expression level of gene in cell lines, each cell line was compared to the expression of its own β actin. High level of *CYP3A4* expression was detected in the cell lines of colorectal carcinoma HCT116, HT-29, HT-29-OxR and myeloid leukemia UT7 compared to the cell lines lung adenocarcinoma A549, ovarian carcinoma A2780, ovarian carcinoma CDDP-resistant subline A2780cis and mammary gland carcinoma T47D. Relative expression level of *CYP3A4* by qRT-PCR analysis in the HT-29, HT-29-OxR, HCT116, A2780, A2780cis, A49, UT7 and T47D cell lines. Relative quantification was used with the comparative CT method, and data were normalised to the housekeeping gene *ß-actin*.

```
Wildtype    GVNIDSLNNPQDPFVENTKKL
HT29        ......................
HT29-OxR    ......F...............
A549        .....FF..SP.......Q..
```

Figure 3. Comparison of deduced partial *CYP3A4* sequences of selected cell lines HT-29, HT-29-OxR, A549. As a wild type an aminoacid sequence of human CYP3A4 protein (GenBank Accession number NP_059488) was used. Dots indicate identical aminoacid.

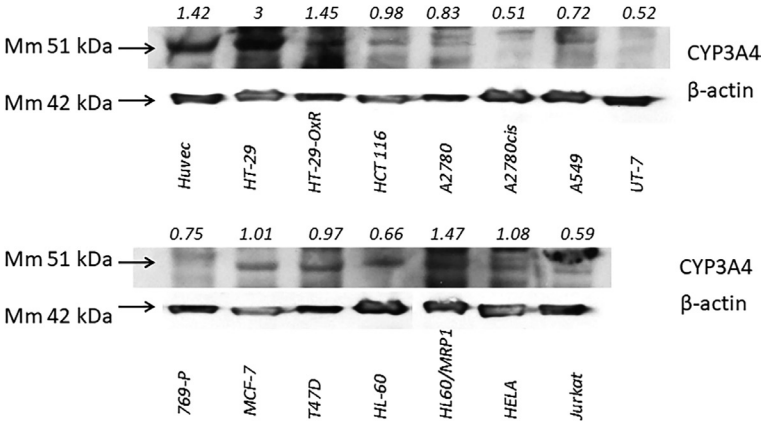

Figure 4. Western blot analysis of CYP3A4. The cell lines: HUVEC, HT-29, HT-29-OxR, HCT116, A2780, A2780cis, A549, UT7, 769-P, MCF7, T47D, HL-60, HL-60/MRP1, Hela, Jurkat. The densitometry of the CYP3A4 proteins was evaluated using ImageJ software and normalized to β-actin protein levels. The optical density is described above each sample.

Results were analyzed using one-way ANOVA with Tukey's *post-hoc* test at three statistical levels: *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. The cell lines were compared mutually with either cell line alone. The comparison of relative gene expression levels was significant between each cell line, with the exception of levels between the two cell lines HT-29 and HT-29-OxR, as well as between cell lines A2780 and T47D. Surprisingly the expression of *CYP3A4* gene in cell line A2780 and its resistant form to cisplatin A2780cis was significant different.

Analysis of CYP3A4 activity under hypericin treatment

The CYP3A4/Luciferin-IPA P450-Glo™ Assay was used to verify whether HYP, HYP-PDT or HP could affect CYP3A4 activity in both parental HT-29 cell line and its resistant form HT-29-OxR (Fig. 6). Interestingly, HYP treatment of HT-29 cells significantly decreased CYP3A4 activity in dark conditions, while HYP-PDT significantly increased CYP3A4 activity. The HYP treatment of HT-29-OxR cells significantly decreased CYP3A4 activity in dark conditions as well as after HYP-PDT, and therefore the resistant cell line was less sensitive to light-activated HYP. In the case of HP, both cell lines responded similarly to treatment and increased CYP3A4 activity, while HT-29-OxR significantly increased CYP3A4 activity after light activation of HP. We can thus conclude that both cell lines responded to the same drugs differently. Although, the CYP activity in both cell lines depends on treatment and type of drugs, both parental and resistant cell lines responded differently to treatment in specific light-activated conditions (Fig. S2).

Discussion

Drugs metabolism is very important for cancer treatment and the selection of an appropriate combination of drugs.

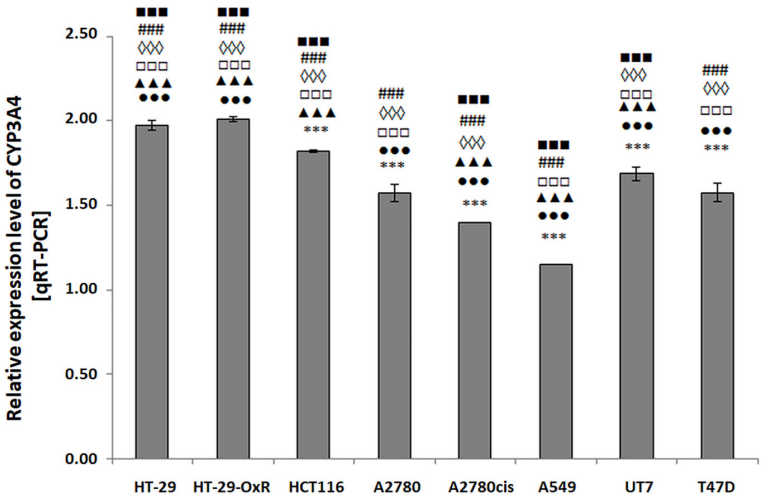

Figure 5. Relative expression level of *CYP3A4* by qRT-PCR analysis in the HT-29, HT-29-OxR, HCT116, A2780, A2780cis, A49, UT7 and T47D cell lines. Relative quantification was used with the comparative CT method, and data were normalised to the housekeeping gene *ß-actin*. Data were processed as means ± SD from three independent experiments. Results were analyzed using one-way ANOVA with Tukey's *post hoc* test at three statistical levels: *** $p < 0.001$. The cell lines were compared mutually with either cell line alone: *** for HT-29 and HT-29-OxR, ●●● for HCT116, ▲▲▲ for A2780, □□□ for A2780cis, ◊◊◊ for A549, ### for UT7, ■■■ for T47D.

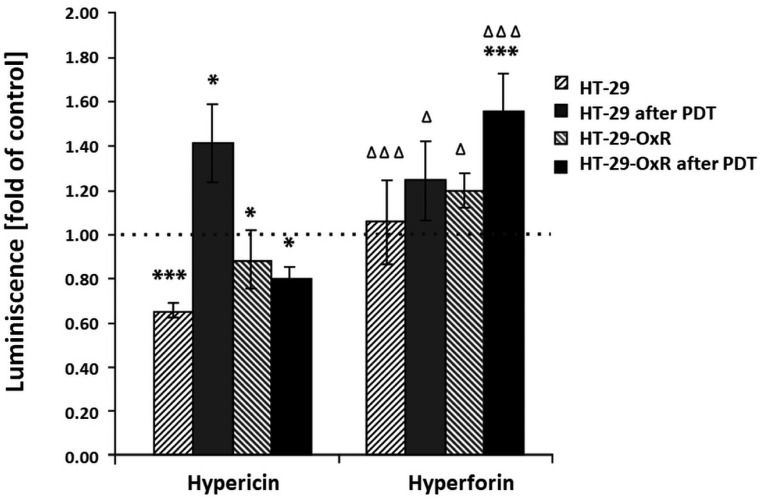

Figure 6. CYP3A4/Luciferin-IPA P450-Glo™ Assays analysis of CYP3A4 activity on HT-29 and HT-29-OxR. The results of experimental groups were analyzed using one-way ANOVA with Tukey's *post-hoc* test. Groups treated with Hypericin or Hyperforin were compared with the control and the experimental groups. The luminiscence content was quantified as the ratio of experimental groups treated with Hypericin and Hyperforin alone compared with control (non affected cells). * $p < 0.05$, *** $p < 0.001$ *vs.* control; $^{\Delta}$ $p < 0.05$, $^{\Delta\Delta\Delta}$ $p < 0.001$ *vs.* Hypericin. The luminescence in controls is shown by dotted line.

The CYP3A4 enzyme is highly inducible by many drugs and dietary chemicals. The principal focus of study was an analysis of *CYP3A4* polymorphism and its influence to variable activity of CYP3A4 proteins and his different effect on the response of HT-29 adenocarcinoma and HT-29-OxR-resistant cancer line to experimental treatment. Moreover, the different level of protein between individual types of cancer cell lines was observed. However, a relationship between the activity of the CYP3A4 protein and the transcription profiles of the CYP3A4 gene of the parental oxaliplatin-resistant HT-29-OxR cell lines compared was found. For the first time, various cancer cell lines were divided using DGGE CYP3A4 transcript profiles. Finally, we found out that DGGE was not sufficient method for study of transcript variants, nevertheless, we can distinguish cell lines based on the *CYP3A4* profile, probably as variant alleles.

Subsequently, sequencing analysis of fragments originated from one isolate confirmed that DGGE fragments of HT-29 had identical sequences, which leads to the hypothesis that the multiband pattern resulted from variant amplicon, similarly as the results of Neilson et al. (2013) and in opposition to the results of Saarikoski et al. (2004), who found different variant alleles of *CYP2S1* using DGGE analysis. Maximum Likelihood (ML) sequences analysis of *CYP3A* family members, individually *CYP3A4, CYP3A5, CYP3A43* and *CYP3A7*, showed the close genetic relation of *CYP3A4* to *CYP3A5*. Surprisingly, a comparison of the same overlapping areas of *CYP3A4* and *CYP3A5* sequences showed variability between tested cell lines.

All our tested cell lines originated from Caucasian ethnicity due to the fact that some CYPs could have different frequency of variant alleles, as for example, the frequency of variant alleles shows differences between ethnic populations with the wild-type *CYP3A5*1* allele more common in Africans than Caucasians and Asians (Bangsi et al. 2006). More than 46 modifications have been identified in the *CYP3A4* gene (http://www.cypalleles.ki.se). Currently, different CYP3A4 variant proteins have been described in the Allele Nomenclature Database, with decreased *(*8,*11,*12,*13,*16A,*16B,*17),* none (**20, *26*) or increased activity *(*18A*) of the allele *in vitro*, whereas an *in vivo* study describes a decrease in two alleles only *(*18A,*22).* Although there is considerable interindividual variation in the expression and activity of CYP3A4 protein, genetic polymorphisms alone do not provide a satisfactory explanation (Rodríguez-Antona et al. 2005). However, their low frequency cannot explain the common interindividual differences in CYP3A4 activity (Bozina et al. 2009). Recently, the results of Zastrozhin et al. (2020) showed the effect of CYP3A4 genetic polymorphisms on alprazolam's efficacy, where the level of CYP3A4 expression correlates with the encoded isoenzyme's activity.

A sequence comparison of amplified *CYP3A4* and *CYP3A5* amplicons with all members of one *CYP3A* family available from GenBank, specifically *CYP3A4, CYP3A5, CYP3A43* and *CYP3A7*, showed the close genetic relation of *CYP3A4* to *CYP3A5* and *CYP3A7*. The comparison confirmed the polymorphism of *CYP3A4* but not *CYP3A5* within the tested cell lines. Single nucleotide polymorphisms (SNPs) represent the most substantial form of genetic variation in humans, which represents more than 90% of all dissimilarities between unrelated individuals. SNP patterns are likely to affect many human phenotypes; thus, extensive studies based on SNP genotyping are await to help identify genes that influence responses to drugs. In addition, the analysis of SNPs may also help adapt drugs to appropriate genotypes, the elementary principle of pharmacogenomics (Wang et al. 2011). More than 30 SNPs were identified in the *CYP3A4* gene. The most common variant

of *CYP3A4* is *CYP3A4*1B* (this is a newly reported SNP in CYP3A4 intron 7 that has been associated in a sex-specific manner with *CYP3A4* expression and enzymatic activity *in vitro*). The studies on pharmacogenetics of drugs were focused on the accesion of the enzyme CYP3A and the drug transporter P-glycoprotein during transplantation of organs. Enzymes and transporters are examined to be the main markers of the pharmacokinetics of currently used immunosuppressive drugs (Yu et al. 2006; Uesugi et al. 2006). Genetic forms attent on polymorphisms in transcription factors (Lamba et al. 2010), different splice variants in transcription factors (He et al. 2005) and differences in regulation of RNA (Pan et al. 2009) have been explained amount of inter-individual variability in CYP3A4 expression or activity. However, how polymorphisms in *CYP3A4* improve variability between individuals in CYP3A4 expression persist unclear (Wang et al. 2009). Due to the genetic polymorphism of CYP3A4*1B was the tacrolimus dose in European (Caucasian) patients characterised as critical (Pan et al. 2009). *CYP3A4*22* establish a danger factor for DGF (delayed graft function) and worsen creatinine clearance in patients after CsA-based immunosuppressive treatment. Therefore, genotyping for the *CYP3A4*22* allele can help identify patients at risk of DGF and low renal function when treated with CsA (Wang et al. 2009). Previous results on the impact of the cytochrome P450 oxidoreductase (POR) as well as other relevant genes (*CYP3A4, CYP3A5, ABCB1*) on variability of tacrolimus pharmacokinetics showed that the POR*28 C>T mutation could reduce the dose-adjusted trough concentrations (C0/D) of tacrolimus in renal recipients (Shi et al. 2015). The recent study of Srinivas et al. (2020) investigated the association of *CYP3A5, CYP3A4* and *ABCB1* gene polymorphisms and their interactions with dose-adjusted tacrolimus trough concentration in blood. The drug-induced showed adverse effects in South Indian renal transplant recipients. The findings of inter-ethnic differences in allele frequencies of drug metabolizer and transporter gene contributes to the need to consider population-specific genetic backgrounds when performing pharmacogenetic analyses and clinical trials.

Several cytochrome P450 (CYP) CYP3A polymorphisms were associated with reduced enzyme function. The study of influence *CYP3A4* mutated allele carriers showed association with diminished drug metabolism capacity. The effect in subjects carrying CYP3A4 double mutations might be relevant due to the majority of subjects lacking the CYP3A5 enzyme. In heterozygous humans, the result might be less visible due to the high potential inducible CYP3A4 (Saiz-Rodríguez et al. 2020). The relationship between polymorphic loci of CYP3A genes (CYP3A4 (rs2740574), CYP3A5 (rs776746) and CYP3A7 (rs2257401) was identified as accompanied to development of chronic mercury intoxication (Chernyak and Merinova 2020).

Polymorphism of DNA was accompanied by low variation at the level of CYP3A4 transcription, when relative expression varied less than 2-fold in tested cell lines. However, a different level of CYP3A4 transcripts was detected in the HT-29 and HT-29-OxR cell lines. Much higher variability was observed on the CYP3A4 protein level, where protein levels varied in the tested cell lines. A very similar high level of CYP3A4 protein was detected in the HUVEC, HT-29-OxR and HL60/MRP-1 cell lines. Interestingly, the level of protein was reduced in resistant form HT-29-OxR compared to its wild type of cell line HT-29 and also in a resistant form A2780cis compared to cell line A2780. The expression of mRNA showed the same conclusions as protein level for A2780 line. All tested cell lines expressed mRNA of CYP3A4 (Fig. 5) in accordance with their protein level (Fig. 4).

The content of CYP3A4 protein and his activity showed inter-individual variability (Elens et al. 2012), influencing drug response and toxicity. Although the expression of *CYP3A4* can be affected by non-genetic factors, genetic factors appears to be associate to inter-individual differences in CYP3A4 activity (Zhang et al. 2015). Genetic factors acting in polymorphisms in transcription factors, different splice variants in transcription factors (Lamba et al. 2002) and differences in RNA regulation (Shimada et al. 1994) reported to account for a portion of interindividual variability in *CYP3A4* expression or activity (Ozdemir et al. 2000).

Drug resistance is a major problem for the treatment of colon cancer, and resistance to oxaliplatin is multifactorial. Many genes have been found to be associated with oxaliplatin resistance (Hector et al. 2001; Boyer et al. 2006). In addition, drug accumulation is also related to resistance, when drug resistance mechanisms include unefficient cellular drug accumulation and uptake (Plasencia et al. 2006) and activation of the antioxidant‘s system for detoxification (Landriscina et al. 2009; Sau et al. 2010).

Finally, the effect of CYP3A4 protein level between two adenocarcinoma cell lines parental and resistant to oxaliplatin (HT-29 and HT-29-OxR) was evaluated in response to drugs treatment, and different activity of CYP3A4 protein was observed, in which the protein level of CYP3A4 in cell line HT-29 was higher compared to that in resistant HT-29-OxR. Two groups of profiles were identified based on the protein level, and thus the cell lines could be distinguished. Similarly, Zhou et al. (2009) recently described that the resistant HT-29 and HCT116 cell lines showed cross-resistance to other chemotherapeutic agents, and changes in cell metabolism were involved in the development of resistance to oxaliplatin in metastatic colorectal cancer cells. This study of protein activity showed a decreased level in cell line HT-29-OxR adenocarcinoma resistant to oxaliplatin and A2780cis resistant cell line compared to its wild type. Therefore, given

the different CYP3A4 protein levels between the cell lines, proteins might affect the efficiency of anti-cancer drugs through their effects on the activity of CYP3A4 and could thus have an impact on metabolism and also resistance to chemotherapy.

Our study shows differences of CYP3A4 protein level among different cancer cell lines, and *CYP3A4* sequences presented different profiles. Moreover, the *CYP3A5* sequences showed variability of the tested cell lines. Finally, HYP and HP treatments demonstrated different CYP3A4 protein activity between the compared cell lines, the parental HT-29 and resistant HT-29-OxR. These results could contribute to evaluation of the activity of cytochrome P450 gene products regarding to their impact on individual cancer therapy.

Acknowledgments. This work was supported by the Slovak Research and Development Agency under the contract and APVV-14-0154. The authors are grateful to David Lee McLean for proofreading the manuscript.

Conflict of interest. The authors declare that there is no competitive and any non-financial conflict of interest in relation to the work described.

References

Agostinis P, Assefa Z, Vantieghem A, Vandenheede JR, Merlevede W, De Witte P (2000): Apoptotic and anti-apoptotic signaling pathways induced by photodynamic therapy with hypericin. Adv. Enzyme Regul. **4,** 157-182 https://doi.org/10.1016/S0065-2571(99)00021-7

Ahn MJ, Park SY, Kim WK, Cho JH, Chang BJ, Kim DJ, Ahn JS, Park K, Han J-S (2012): A single nucleotide polymorphism in the phospholipase D1 gene is associated with risk of non-small cell cancer. Int. J. Biomed. Sci. **8,** 121-128

Avril T, Vauléon E, Chevet E (2017) Endoplasmic reticulum stress signaling and chemotherapy resistance in solid cancers. Oncogenesis **6,** e373 https://doi.org/10.1038/oncsis.2017.72

Bangsi D, Zhou J, Sun Y, Patel NP, Darga LL, Heilbrun LK, Powell IJ, Severson RK, Everson RB (2006): Impact of a genetic variant in CYP3A4 on risk and clinical presentation of prostate cancer among white and African-American men. Urol. Oncol. **24,** 21-27 https://doi.org/10.1016/j.urolonc.2005.09.005

Boyer J, Allen WL, McLean EG, Wilson PM, McCulla A, Moore S, Longley DB, Caldas C, Johnston PG (2006): Pharmacogenomic identification of novel determinants of response to chemotherapy in colon cancer. Cancer Res. **66,** 765-777 https://doi.org/10.1158/0008-5472.CAN-05-2693

Bozina N, Bradamante V, Lovrić M (2009): Genetic polymorphism of metabolic enzymes P450 (CYP) as a susceptibility factor for drug response, toxicity, and cancer risk. Arh. Hig. Rada Toksikol. **60,** 217-242 https://doi.org/10.2478/10004-1254-60-2009-1885

Cascorbi I (2012): Drug interactions-principles, examples and clinical consequences. Dtsch. Arztebl. Int. **109,** 546-555 https://doi.org/10.3238/arztebl.2012.0546

Elens L, Bouamar R, Hesselink DA, Haufroid V, van Gelder T, van Schaik RH (2012): The new CYP3A4 intron 6 C>T polymorphism (CYP3A4*22) is associated with an increased risk of delayed graft function and worse renal function in cyclosporine-treated kidney transplant patients. Pharmacogenet. Genomics **22,** 373-380 https://doi.org/10.1097/FPC.0b013e328351f3c1

Erdelmeier CA (1998): Hyperforin possibly the major non-nitrogenous secondary metabolite of Hypericum perforatum L. Pharmacopsychiatry **31,** 2-6 https://doi.org/10.1055/s-2007-979339

Fletcher J, Haber M, Henderson M, Norris D (2010): ABC transporters in cancer: more than just drug efflux pumps. Nat. Rev. Cancer **10,** 147-156 https://doi.org/10.1038/nrc2789

Gottesman M (2002): Mechanisms of cancer drug resistance. Annu. Rev. Med. **53,** 615-627 https://doi.org/10.1146/annurev.med.53.082901.103929

Guldberg P, Grønbœk K, Worm J, thor Straten P, Zeuthen J (2002): Mutational analysis of oncogenes and tumor suppressor genes in human cancer using Denaturing Gradient Gel Electrophoresis molecular analysis of cancer. Methods Mol. Med. **68,** 125-139 https://doi.org/10.1385/1-59259-135-3:125

He P, Court MH, Greenblatt DJ, Von Moltke LL (2005): Genotype-phenotype associations of cytochrome P450 3A4 and 3A5 polymorphism with midazolam clearance in vivo. Clin. Pharmacol. Ther. **77,** 373-387 https://doi.org/10.1016/j.clpt.2004.11.112

Hector S, Bolanowska-Higdon W, Zdanowicz J, Hitt S, Pendyala L (2001): In vitro studies on the mechanisms of oxaliplatin resistance. Cancer Chemother. Pharmacol. **48,** 398-406 https://doi.org/10.1007/s002800100363

Holmila R, Husgafvel-Pursiainen K (2006): Analysis of TP53 gene mutations in human lung cancer: comparison of capillary electrophoresis single strand conformation polymorphism assay with denaturing gradient gel electrophoresis and direct sequencing. Cancer Detect. Prev. **30,** 1-6 https://doi.org/10.1016/j.cdp.2005.10.004

Hoya M, Díaz-Rubio E, Caldés T (1999): Denaturing gradient gel electrophoresis-based analysis of loss of heterozygosity distinguishes nonobvious, deleterious BRCA1 variants from nonpathogenic polymorphisms. Clin. Chem. **45,** 2028-2030 https://doi.org/10.1093/clinchem/45.11.2028

Huttunen KM, Mahonen N, Raunio H, Rautio J (2008): Cytochrome P450-activated prodrugs: targeted drug delivery. Curr. Med. Chem. **15,** 2346-2365 https://doi.org/10.2174/092986708785909120

Chernyak YuI, Merinova AP (2020): CYP3A polymorphism and chronic mercury intoxication. Bull. Exp. Biol. Med. **168,** 492-495 https://doi.org/10.1007/s10517-020-04738-4

Kozubík A, Vaculová A, Souček K, Vondráček J, Turánek J, Hofmanová J (2008): Novel anticancer platinum (IV) complexes

with adamantylamine: their efficiency and innovative chemotherapy strategies modifying lipid metabolism. Met. Based Drugs **2008,** 417897
https://doi.org/10.1155/2008/417897

Lamba JK, Lin YS, Schuetz EG, Thummel KE (2002): Genetic contribution to variable human CYP3Amediated metabolism. Adv. Drug Deliv. Rev. **54,** 1271-1294
https://doi.org/10.1016/S0169-409X(02)00066-2

Lamba V, Panetta JC, Strom S, Schuetz EG (2010): Genetic predictors of interindividual variability in hepatic CYP3A4 expression. J. Pharmacol. Exp. Ther. **332,** 1088-1099
https://doi.org/10.1124/jpet.109.160804

Landriscina M, Maddalena F, Laudiero G, Esposito F (2009): Adaptation to oxidative stress, chemoresistance, and cell survival. Antioxid. Redox. Signal. **11,** 2701-2716
https://doi.org/10.1089/ars.2009.2692

Lee SJ, Goldstein JA (2005): Functionally defective or altered CYP3A4 and CYP3A5 single nucleotide polymorphisms and their detection with genotyping tests. Pharmacogenomics **6,** 357-371
https://doi.org/10.1517/14622416.6.4.357

Neilson JW, Jordan FL, Maier RM (2013): Analysis of artifacts suggests DGGE should not be used for quantitative diversity analysis. J. Microbiol. Methods **92,** 256-263
https://doi.org/10.1016/j.mimet.2012.12.021

Oyama T, Kagawa N, Kunugita N, Kitagawa K, Ogawa M, Yamaguchi T, Suzuki R, Kinaga T, Yashima Y, Ozaki S, et al. (2004): Expression of cytochrome P450 in tumor tissues and its association with cancer development. Front. Biosci. **9,** 1967-1976
https://doi.org/10.2741/1378

Ozdemir V, Kalow W, Tang BK, Paterson AD, Walker SE, Endrenyi L, Kashuba AD (2000): Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. Pharmacogenetics **10,** 373-388
https://doi.org/10.1097/00008571-200007000-00001

Pan YZ, Gao W, Yu AM (2009): MicroRNAs regulate CYP3A4 expression via direct and indirect targeting. Drug. Metab. Dispos. **37,** 2112-2117
https://doi.org/10.1124/dmd.109.027680

Pharoah PDP, Tyrer J, Dunning A, Easton DF, Ponder BAJ (2007): Association between common variation in 120 candidate genes and breast cancer risk. PLoS Genet. **3,** e42
https://doi.org/10.1371/journal.pgen.0030042

Plasencia C, Martinez-Balibrea E, Martinez-Cardus A., Quinn DI, Abad A, Neamati N (2006): Expression analysis of genes involved in oxaliplatin response and development of oxaliplatin-resistant HT-29 colon cancer cells. Int. J. Oncol. **29,** 225-235
https://doi.org/10.3892/ijo.29.1.225

Rodríguez-Antona C, Sayi JG, Gustafsson LL, Bertilsson L, Ingelman-Sundberg M (2005): Phenotype-genotype variability in the human CYP3A locus as assessed by the probe drug quinine and analyses of variant CYP3A4 alleles. Biochem. Biophys. Res. Commun. **338,** 299-305
https://doi.org/10.1016/j.bbrc.2005.09.020

Saarikoski ST, Suitiala T, Holmila R, Impivaara O, Järvisalo J, Hirvonen A, Husgafvel-Pursiainen K (2004): Identification of genetic polymorphisms of CYP2S1 in a Finnish caucasian population. Mutat. Res. **54,** 267-277
https://doi.org/10.1016/j.mrfmmm.2004.05.007

Saiz-Rodríguez M, Almenara S, Navares-Gómez M, Ochoa D, Román M, Zubiaur P, Koller D, Santos M, Mejía G, Borobia AM et al. (2020): Effect of the most relevant CYP3A4 and CYP3A5 polymorphisms on the pharmacokinetic parameters of 10 CYP3A substrates. Biomed. **8,** E94
https://doi.org/10.3390/biomedicines8040094

Sau A, Pellizzari TF, Valentino F, Federici G, Caccuri AM (2010): Glutathione transferases and development of new principles to overcome drug resistance. Arch. Biochem. Biophys. **500,** 116-122
https://doi.org/10.1016/j.abb.2010.05.012

Shi WL, Tang HL, Zhai SD (2015): Effects of the CYP3A4*1B Genetic polymorphism on the pharmacokinetics of tacrolimus in adult renal transplant recipients: A meta-analysis. PLoS One **10,** e0127995
https://doi.org/10.1371/journal.pone.0127995

Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP (1994): Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J. Pharmacol. Exp. Ther. **270,** 414-423

Srinivas L, Gracious N, Nair RR (2020): Sun-312 insights into the association of CYP3A and ABCB1 polymorphisms with tacrolimus-related adverse effects - venturing beyond the conventional SNP-Tac level relationship. Kidney Int. Rep. **5,** S330
https://doi.org/10.1016/j.ekir.2020.02.849

Straková N, Hofmanová J, Souček K, Fedr R, Hyršlová Vaculová A, Zapletal O, Tylichová Z, Lauková J, Bouchal J, et al. (2013): Oxaliplatin with PPARγ ligand modulate regulation of the cell cycle and cell death of colon tumors. The 9th Symposium & Workshop on Molecular Pathol. Histo(cyto)chemistry, p.20

Sun YL, Patel A, Kumar P, Chen ZS (2012): Role of ABC transporters in cancer chemotherapy. Chin. J. Cancer **31,** 51-57
https://doi.org/10.5732/cjc.011.10466

Szatmari I, Vámosi G, Brazda P, Balint L, Benko S, Széles L, Viktoria Jeney V, Ozvegy-Laczka C, Szántó A, Barta E et al. (2006): Peroxisome proliferator-activated receptor gamma-regulated ABCG2 expression confers cytoprotection to human dendritic cells. J. Biol. Chem. **281,** 23812-23823
https://doi.org/10.1074/jbc.M604890200

Šemeláková M, Mikeš J, Jendželovský R, Fedoročko P (2012): The pro-apoptotic and anti-invasive effects of hypericin-mediated photodynamic therapy are enhanced by hyperforin or aristoforin in HT-29 colon adenocarcinoma cells. J. Photochem. Photobiol. B **117,** 115-125
https://doi.org/10.1016/j.jphotobiol.2012.09.003

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. **30,** 2725-2729
https://doi.org/10.1093/molbev/mst197

Uesugi M, Masuda S, Katsura T, Oike F, Takada Y, Inui K (2006): Effect of intestinal CYP3A5 on postoperative tacrolimus trough levels in living-donor liver transplant recipients. Pharmacogen. Genomics **16,** 119-127
https://doi.org/10.1097/01.fpc.0000184953.31324.e4

Varinská L, Fáber L, Kello M, Petrovová E, Balážová Ľ, Solár P, Čoma M, Urdzík P, Mojžiš J, Švajdlenka E et al. (2018): β-escin effectively modulates HUVECS proliferation and tube formation. Molecules **23,** 197

https://doi.org/10.3390/molecules23010197
Wang D, Guo Y, Wrighton SA, Cooke GE, Sadee W (2011): Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. Pharmacogenomics J. **11,** 274-286
https://doi.org/10.1038/tpj.2010.28
Wang Y, Wang C, Li J, Wang X, Zhu G, Chen X, Bi H, Huang M (2009): Effect of genetic polymorphisms of CYP3A5 and MDR1 on cyclosporine concentration during the early stage after renal transplantation in Chinese patients co-treated with diltiazem. Eur. J. Clin. Pharmacol. **65,** 239-247
https://doi.org/10.1007/s00228-008-0577-4
Westlind-Johnsson A, Malmebo S, Johansson A, Otter C, Andersson TB, Johansson I, Edwards RJ., Boobis AR., Ingelman-Sundberg M (2003): Comparative analysis of CYP3A expression in human liver suggests only a minor role for CYP3A5 in drug metabolism. Drug Metab. Dispos. **31,** 755-761
https://doi.org/10.1124/dmd.31.6.755
Wijnen J, Fodde R, Khan PM (1994): DGGE polymorphism in intron 10 of MSH2, the HNPCC gene. Human Mol. Genet. **3,** 2268
https://doi.org/10.1093/hmg/3.12.2268-a
Xu C, Li CY, Kong AN (2005): Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch. Pharm. Res. **28,** 249-268
https://doi.org/10.1007/BF02977789
Yu RZ, Geary RS, Levin AA (2006): Pharmacokinetics and pharmacodynamics of antisense oligonucleotides. In: Pharmacokinetics and Pharmacodynamics of Biotech Drugs: Principles and Case Studies in Drug Development. (Ed. B. Meibohm), pp. 93-120, Wiley-VCH Verlag
https://doi.org/10.1002/9783527609628.ch4
Zastrozhin MS, Skryabin VY, Smirnov VV, Petukhov AE, Pankratenko EP (2020): Effects of plasma concentration of microRNA Mir-27b and CYP3A4*22 on equilibrium concentration of alprazolam in patients with anxiety disorders comorbid with alcohol use disorder. Gene **739,** 144513
https://doi.org/10.1016/j.gene.2020.144513
Zhang JJ, Liu SB, Xue L, Ding XL, Zhang H, Miao LY (2015): The genetic polymorphisms of POR*28 and CYP3A5*3 significantly influence the pharmacokinetics of tacrolimus in Chinese renal transplant recipients. Int. J. Clin. Pharmacol. Ther. **53,** 728-736
https://doi.org/10.5414/CP202152
Zhou SF, Liu JP, Chowbay B (2009): Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug. Metab. Rev. **41,** 89-295
https://doi.org/10.1080/03602530902843483

Received: October 25, 2019
Final version accepted: December 15, 2020

doi: 10.4149/gpb_2020043

Supplementary Material

CYP3A gene variability and cancer cells response to the treatment

Martina Šemeláková[1], Lenka Maliničová[2], Mária Macejová[2] and Peter Pristaš[2]

[1] *Department of Medical Biology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovakia*
[2] *Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Košice, Slovakia*

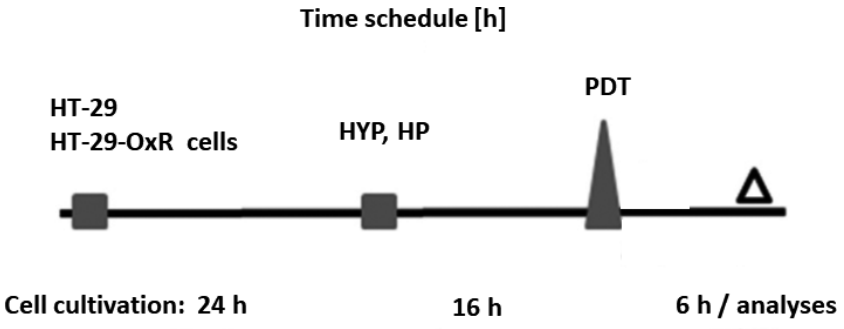

Figure S 1. PDT treatment scheme. HT-29 and HT-29-OxR cells were incubated for 24 h before treatment with non-activated HYP or HP. Cells were treated with PDT (3.15 J/cm^2) at 16 h after treatment. Analyses were performed at time 6 h after PDT.

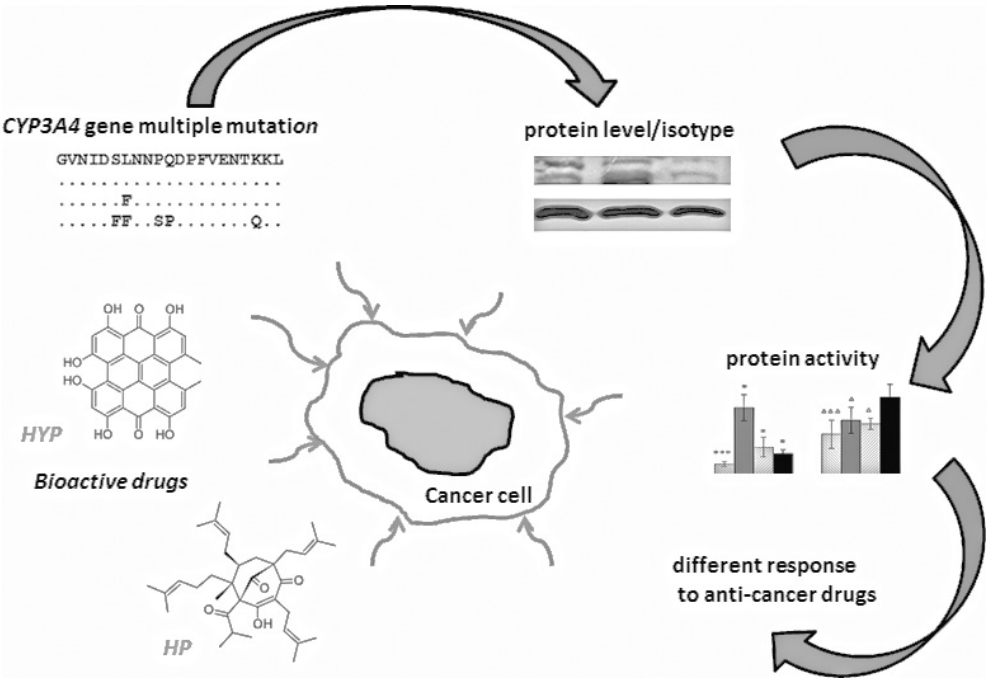

Figure S 2. The effect of the bioactive drugs HYP and HP on the modification of CYP3A4 activity in HT-29 and HT-29-OxR cell lines in the treatment of cancer, and mutations in CYP3A4 genes, different mRNA expression and protein levels between different cancer cell lines were identified.