

Abstract

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Title of doctoral thesis: Novel approaches for development of *in vitro* liver cell models.

The liver is a main metabolizing organ in the body. Therefore, the evaluation of hepatic metabolism is a crucial step during drug development. Moreover, a liver damage induced by drugs is another task to be assessed in drug development. *In vitro* liver cell models allow addressing some of these concerns. Up to date, primary human hepatocytes are considered as a “gold” standard of *in vitro* liver cell models. Additionally, other liver model systems are used such as liver tissue slices, subcellular fractions, liver cancer cell lines and hepatocytes derived from stem cells. Despite the significant progress towards right estimation of pharmacokinetic and toxicological parameters of drug candidates during drug development, current *in vitro* systems still suffer from various drawbacks. One of these limitations is their insufficient similarity with *in vivo*-like phenotype associated with low metabolic capacity of the models. In last several years, we were victims of tremendous effort to improve existing models such as 3D models, co-culture and perfusion cultures.

Aims of this thesis can be divided into three parts. Firstly, we tried to enhance metabolic capacity of *in vitro* liver cell models using small molecules, which are activators of crucial nuclear receptors controlling expression of drug-metabolizing enzymes (DMEs). Liver cancer cell lines were predominantly utilized since these cells have fast and unlimited life-span, good availability and easy handling but lack many DMEs. Secondly, we focused on the impact of post-transcriptional and post-translational modifications of key regulators of DMEs, which could also shed some light on the phenomenon of low DMEs expression in common liver cell lines. Finally, we applied *in vitro* liver cell models including stem cell-derived hepatocytes for a hepatotoxicity assessment of newly developed drug candidates and suspected hepatotoxicans-derived from medical plants.

In our first study, we identified MEK1/2 inhibitors as strong activators CYP3A subfamily genes in commonly used hepatocellular carcinoma cell line HepG2. These results are important since CYP3A4 isoform metabolizes more than 30% of all clinically used drugs and it is poorly expressed in this cell line as well as in other liver cell models. Furthermore, we attempted to decipher the mechanism underlying this phenomenon.

Secondly, we identified several new compounds functioning as “pan-xenosensors” ligands. Since nuclear receptors (NRs) such as PXR, CAR and transcription factor AhR are involved in the gene regulation of DMEs, we suppose that these small molecules can be used for the boosting of DME gene expression in *in vitro* liver models.

In another study, we focused on *in silico* prediction of microRNAs (miRNAs) targeting NRs, which are associated with CYP3A4 regulation. We and other groups suggested that the effect of miRNAs on the regulation of NRs could participate in CYP3A4 interindividual expression variability. We can hypothesize that our predicted miRNAs could have an impact on the expression of DMEs in *in vitro* liver cell models.

The better knowledge about the regulatory network controlling PXR (and other NRs) functions can lead to possible modifications of *in vitro* liver models allowing to optimize their properties closer to *in vivo*-like phenotype.

In our next study, we tried to estimate a toxicity of newly synthesized compounds with a potential antituberculosic activity. To fulfill the aim of the study, we used primary human hepatocytes and four different mammalian cell lines including hepatic HuH-7 and HepG2 cells. The mitochondrial activity measured by MTS assay was selected as a marker of a cell viability. Our tested molecules revealed generally low cellular toxicity with several exceptions.

Currently, we evaluate induced hepatocyte-like cells (iHep cells) for toxicological screening in our preparing manuscript (not yet published). For this analysis, we used several hepatotoxic phytochemicals with different mode of toxicity to validate model sensitivity and compared iHep cells with HepG2 cells and primary hepatocytes. We believe that such analysis can bring a reference point for tracking of a position of currently available iHep cells for toxicological applications.

Collectively, our investigation brought some novel small molecules used for the adjustment metabolic capacity of current *in vitro* liver models. Moreover, we

described some aspects of post-transcriptional and post-translational modifications involved in DME regulation. Finally, we applied *in vitro* cell models for the prediction of hepatotoxicity of newly synthesized drug candidates with a potential antituberculous activity and suspected hepatotoxicants-derived from medical plants.