

Transcriptomics close to my heart

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• • • Addendum I: Valorization • • •

Valorization of scientific knowledge is “the process of creating value from knowledge, by making knowledge suitable and/or available for social and/or economic use and by making knowledge suitable for translation into competitive products, services, processes and new commercial activities”^[1]. The opportunities for valorization of the current dissertation are described in this chapter.

Relevance

Drug development and safety assessment are long and costly processes, which are currently not able to eliminate all health risks related to drug use^[2]. This thesis aimed to improve toxicological drug safety assessments by adopting an experimental *in vitro* design better reflecting the human *in vivo* conditions combined with advanced analysis of post-transcriptional mechanisms.

Because current *in vitro* toxicological drug safety assessment is unable to detect all drug-induced toxicities, many investigated candidate compounds fail during animal testing and never enter the market^[3]. Animal experiments are time consuming, labor intensive and very expensive^[4]. Therefore, failure of a candidate compound due to toxicities is a waste of money and resources which can be prevented through improved *in vitro* assays. But even when a candidate compound passes all stages of the safety assessment, side effects or adverse drug reactions can occur due to undetected toxicities. Animal experiments are usually performed using mice or rats and toxicity results are extrapolated to predict human toxicities^[5]. Overall, the accuracy of human risk prediction using rodent experiments is only 50-70%, which is barely better than flipping a coin^[6]. The adverse drug reactions are a major cause for regulatory actions like restrictions on drug use in the form of black box warnings or even drug withdrawal from the market^[7-9]. This again results in an economical burden in the form of money and resources squandered, but also increase ethical objections to animal testing. More important is the societal impact of incorrect human risk assessments because drug induced toxicities rank high as cause of disease and death^[10]. This clearly indicates that there is need for improvement of drug safety testing, to which the results in this thesis contribute.

Target Groups

While the outcome of this thesis may be relevant for a diverse range of scientific fields, we focus on the field of toxicology. Target groups for whom the outcome of this thesis may be of interest include regulatory agencies, industry and ultimately the general population.

Specialized regulatory organizations (i.e. U.S Food and Drug Administration; FDA) are responsible for the safety of pharmaceuticals, food-additives and chemicals. For monitoring purposes, strict guidelines are developed which contain regulations and recommendations regarding the safety testing procedure^[11,12]. Before new *in vitro* test methods can be implemented in toxicological safety assessments, they need to be approved by these regulatory agencies. In this context, the models and methods described in this thesis may be of interest to these regulatory organizations.

All branches of industry dealing with toxicological testing of compounds may benefit from our results. While this also includes manufactures of food products and cosmetics, the highest relevance will be for the chemical and pharmaceutical companies. The ability to reliably predict human risk *in vitro* will have tremendous benefits for these industries. Because the toxicity of a candidate compound can be assessed during early phases of the drug development process, money and resources (including animals) can be saved for compounds with actual therapeutic potential. Furthermore, this would also lead to fewer animal experiments. Therefore, the results in this thesis also contribute towards the replacement, reduction and refinement of animal testing^[13], which eases the societal resistance against animal testing. Furthermore, reliable risk assessment would prevent incorrect conclusions. This not only prevents costly drug withdrawal from the market, but also prevents compounds from being wrongfully classified as toxic. These additional non-toxic compounds with therapeutic potential increases the chance of finding new treatments. Overall, better *in vitro* testing methods for toxicological assessment, would make the drug development and safety

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assessment processes more efficient and cheaper. Subsequently, prices could be decreased for medication and health care in general, which could the general population.

Activities and Innovation

In the first part of this thesis, we described the use of 3D cell cultures exposed to PBPK-based dosing profiles to reflect more accurately the *in vivo* human responses to a toxicant. Current toxicological exposures are usually performed using a stable toxicant dose, often at higher concentrations than found *in vivo* to strengthen the toxic effects for investigation. In **Chapter 3** we showed the difference in cellular responses between the therapeutic and the toxic dosing profile of doxorubicin, thereby highlighting the importance of investigating relevant *in vivo* therapeutic doses during toxicological assessments. Additionally, our innovative experimental design incorporates influences of toxicant absorption, distribution, metabolism and excretion through changing of toxicant doses (three times daily) based on PBPK-models. While this procedure increases the labor intensity of *in vitro* exposures, it highly contributes to creating resemblance of *in vitro* models to *in vivo* situations. Therefore, the accuracy of *in vitro* human toxicological risk assessment could be increased by adopting such *in vitro* models. Furthermore, in **Chapter 2** we evaluated the effect of 0.1% DMSO exposure and showed for the first time that DMSO does not only alter gene expression, but also more strongly influences DNA methylation patterns of human cells *in vitro*. While a concentration of 0.1% DMSO is generally viewed as a low concentration, it is actually extremely high compared to physiologically relevant *in vitro* toxicant doses. For example, the maximum toxicant concentration used in the therapeutic DOX-dosing profile applied in this thesis was 0.22 μM , while 0.1% DMSO equals a concentration of 14.1 mM! This implies that the generally used concentration of 0.1% DMSO which may pose a threat in generating erroneous conclusions gained from *in vitro* toxicity assessments (i.e. false negative drug toxicity conclusions). Therefore, use of DMSO during *in vitro* assays should be avoided or reduced where possible.

The second part of this thesis focused on using high throughput sequencing and advanced data-analysis to obtain a deeper understanding of drug-induced toxicity. While the use of sequencing technology to analyze changes in gene expression already enables the complete monitoring of toxicant-induced effects in a cell, there is more detailed information to be gained from RNA sequencing data through incorporating advanced data-analysis of post-transcription mechanisms.

In **Chapter 4** we showed the added value of analyzing toxicant-induced changes due to alternative splicing through which one gene can encode a great variety of transcripts which differ in biological functions as well as their ability to be translated into a protein. During the analysis of anthracycline- exposed samples, we observed changes in ratios of expressed transcripts (defined as differential transcript usage; DTU) which entailed a switch from a protein-coding transcript to a non-protein coding transcript. While gene expression was not significantly changed, the observed decrease in protein coding transcripts implied a decrease of protein content which may contribute to the cardiotoxic effects of anthracyclines. Therefore, analysis of DTU provides an extra layer of detail able to identify toxicant-induced changes, which would have been overlooked when solely analyzing gene expression changes. Furthermore, in **Chapter 5** we designed a scoring system to identify changes in the post-transcriptional microRNA-induced inhibition of translation. The scoring system was designed based on mRNA, microRNA and circular RNA expression values and predicted interactions.

Compared to unadjusted RNA counts, estimated translatable mRNA read counts gained using the scoring system correlate better with the obtained proteome data, which are the functional entities of the cell. Although the results of this analysis are still preliminary, this suggests the potential of applying the scoring system to the whole transcriptome in order to estimate the protein abundance of proteins which could not be measured using proteomics techniques due to its detection limit. This would result in a complete dataset which is more relevant for evaluating functions and the investigated phenotype.

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These new approaches incorporating assessment of post-transcriptional mechanisms should service the further enrichment of analytical tools that will become necessary in future research to make optimal use of sequencing data to allow a better understanding of which compounds cause toxicity and what mechanisms are affected.

Implementation

In this thesis we described an experimental design better reflecting the human *in vivo* conditions combined with advanced analysis of post-transcriptional mechanisms. Using these models and methods, an increased understanding of cellular processes affected by a toxicant can be obtained. This information may be applied for the identification of novel biomarkers for early detection of toxicity or even to identify new treatment targets to prevent toxic phenotypes. However, it would be more beneficial to incorporate this more accurate *in vitro* test directly into safety testing procedures to more reliably predict the *in vivo* human responses to a toxicant. Though the research community has embraced the power of high throughput omics analysis, regulatory agencies have not yet acknowledged its value for toxicological assessments. Regulators still remain skeptical about the reliability and robustness of data gained from omics analysis, mainly due to inter-platform variations and bioinformatics pipeline-dependent differences. In order to address these issues, a new project has been created: “Towards the Development of an Omics Data Analysis Framework (ODAF) for Regulatory Application” (CEFIC-LRI-C4)^[14]. In this project, we will develop a regulatory ODAF (R-ODAF) to enable the regulatory bodies to consider omics data as a relevant data type for the purpose of supporting toxicological drug safety assessments.

References

- 1 Maastricht promotie reglement. (2018).
- 2 Verheijen, M., Krauskopf, J., Kleinjans, J. C., de Kok, T. M. & Caiment, F. Development and regulatory application of microRNA biomarkers. *Biomark Med* **9**, 1137-1151, doi:10.2217/bmm.15.50 (2015).
- 3 Roberts, R. A. *et al.* Reducing attrition in drug development: smart loading preclinical safety assessment. *Drug discovery today* **19**, 341-347, doi:10.1016/j.drudis.2013.11.014 (2014).
- 4 Levy, N. The use of animal as models: ethical considerations. *Int J Stroke* **7**, 440-442, doi:10.1111/j.1747-4949.2012.00772.x (2012).
- 5 Walmsley, R. M. & Billinton, N. How accurate is in vitro prediction of carcinogenicity? *British Journal of Pharmacology* **162**, 1250-1258, doi:10.1111/j.1476-5381.2010.01131.x (2011).
- 6 Olson, H. *et al.* Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul Toxicol Pharm* **32**, 56-67, doi:10.1006/rtp.2000.1399 (2000).
- 7 Chen, M. *et al.* Toward predictive models for drug-induced liver injury in humans: are we there yet? *Biomark Med* **8**, 201-213, doi:10.2217/bmm.13.146 (2014).
- 8 Osaki, M., Kosaka, N., Okada, F. & Ochiya, T. Circulating microRNAs in drug safety assessment for hepatic and cardiovascular toxicity: the latest biomarker frontier? *Mol Diagn Ther* **18**, 121-126, doi:10.1007/s40291-013-0065-0 (2014).
- 9 Hornberg, J. J. *et al.* Exploratory toxicology as an integrated part of drug discovery. Part I: Why and how. *Drug discovery today* **19**, 1131-1136 (2014).
- 10 Hornberg, J. J. *et al.* Exploratory toxicology as an integrated part of drug discovery. Part II: Screening strategies. *Drug discovery today* **19**, 1137-1144, doi:10.1016/j.drudis.2013.12.009 (2014).
- 11 *Guidance on REACH (Registration Evaluation Authorization and restriction of Chemicals)*, <<https://echa.europa.eu/guidance-documents/guidance-on-reach>> (accessed on Oct 2018).
- 12 *(Organisation for Economic Co-operation and Development) OECD Test Guidelines for the Chemicals*, <<http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm>> (accessed on Oct 2018).
- 13 Baumans, V. Use of animals in experimental research: an ethical dilemma? *Gene therapy* **11**, S64 (2004).
- 14 Caiment, F. LRI C4 – Towards the Development of an Omics Data Analysis Framework (ODAF) for Regulatory Application, <<http://cfic-lri.org/request-for-proposals/lri-c4-towards-the-development-of-an-omics-data-analysis-framework-odaf-for-regulatory-application/>> (accessed on Oct 2018).