Integrating cellular and tissue dynamics with cell fate decisions through computational modeling

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English Summary

Animal testing remains a necessary step during preclinical drug safety studies, but alternative approaches that substitute and thereby reduce the use of animals are gaining ground. Experimental *in vitro* methods with human-derived cell lines can be used to monitor the effect of chemicals on individual living cells. Under normal conditions, cells are in homeostasis and express proteins at a basal level. Upon exposure to cytotoxic chemicals, diverse types of damage lead to the activation of stress response pathways, such as the DNA damage response (DDR), oxidative stress response (OSR) and unfolded protein response (UPR). These signaling pathways play a role in various processes that limit the amount of cellular damage, help cells to recover or orchestrate irreversible cell fates such as senescence and apoptosis. Low amounts of damage are usually surmountable, but require activation of a specific set of proteins that resolve the damage. In addition, cells need time to recover, thus preventing continued damage within daughter cells. This highlights the importance of adequate cell cycle regulation, which should depend on stress and damage levels.

With live-cell microscopy, the above-mentioned processes can be monitored in individual living cells over time. Human cell lines are particularly useful for live-cell microscopy, because they can be propagated indefinitely and manipulated in such a way that the extent of intracellular protein expression becomes visible under the microscope. This allows to monitor protein dynamics and cell fate in different exposure scenarios. However, such an experimentally obtained characterization of cellular processes upon chemical exposure is mainly qualitative. In contrast, quantitative, *in silico* descriptions of the observed protein dynamics and their role in the onset of adverse cellular reactions can help to elucidate the mechanisms underlying adversity and can be used to generate predictions for similar conditions. Moreover, processes that take place on different levels of biological organization can be coupled in a single quantitative framework using mathematical models that describe biochemical

reactions and cell fate. Computational approaches are complementary to experimental methods as they can provide a simplified albeit detailed understanding of complex biological systems.

The signaling pathways that regulate cell fates such as cell cycle progression, senescence and apoptosis have been extensively studied and many proteins that play pivotal roles in these processes have been identified. In this thesis, we use experimental data and current knowledge on stress pathway activation and cell fate to create different types of computational models. With these models, we mathematically describe intracellular protein signaling cascades activated upon exposure to various compounds and their link to cell fate. In this way, we integrate molecular-level biological processes to cell-level phenomena such as cell cycle progression, senescence and necrosis, and generate new hypotheses about the mechanisms underlying adversity.

As a first step, we create an ordinary differential equation (ODE) model for the DNA damage response upon exposure to cisplatin (a frequently used chemotherapeutic agent inducing DNA damage) in the hepatocellular carcinoma cell line HepG2 to quantitatively describe the effect of genotoxicity on intracellular protein dynamics (**Chapter 2**). We model the activation of the central regulator of the DDR, the transcription factor p53, and three of its downstream targets MDM2, p21 and BTG2. This model is calibrated to data originating from HepG2 cells that have different enzyme and transporter expression levels than healthy hepatocytes, and behave differently from cells in human liver tissue. However, equally high-content data is not available for cells in healthy liver tissue, so the applicability of this model for healthy cells is unknown. Therefore, we investigate whether this HepG2-based model can be used to predict pathway activation in primary human hepatocytes (PHHs), i.e., cells that originate from healthy liver tissue. We use mRNA transcription data of the genes that encode p53, MDM2, p21 and BTG2, i.e., *TP53*, *MDM2*, *CDKN1A* and *BTG2* respectively, in 50 PHH donor samples to determine the correlation between the expression of *TP53* and each of the downstream targets. In addition, we use our computational model to create 50 virtual donor samples and compare the model-based gene correlations with the gene correlations found in PHHs. Our model predicts equally low positive correlations between *TP53* and *CDKN1A*, and *TP53* and *BTG2* as observed in PHHs, but cannot reproduce the slightly negative correlation between *TP53* and *MDM2* in PHHs. With this study, we thus show that the p53-p21 and p53-BTG2 relation in PHHs is well-explained by HepG2 cells. However, either the p53-MDM2 relation in HepG2 cells deviates from PHHs, or our model is missing essential interactions to explain the negative relation between *TP53* and *MDM2* expression.

Despite the incongruence between our model and PHHs concerning the p53-MDM2 relation, the description of protein expression dynamics in HepG2 cells is highly accurate. Therefore, we use the same model as a base to study the link

between protein expression and cell cycle arrest (**Chapter 3**). For this purpose, we create an elementary cell cycle progression model that simulates the number of cells in the distinct cell cycle phases over time. With this model, we investigate the role of cell cycle regulators p21 and BTG2 in the induction of the G2 cell cycle arrest that occurs upon exposure to the chemotherapeutic drugs cisplatin and etoposide. We find that BTG2 expression alone is sufficient to explain the cisplatin-induced, but not the etoposide-induced G2 arrest. A model with continued cell cycling from G2 phase to G1 without mitosis in combination with altered cell cycle phase transition rates can explain the etoposide data. In addition, cell cycle continuation following the transient G2 arrest appears unrelated to p21 and BTG2 expression, because the expression of these proteins remains high. In conclusion, we show that the expression dynamics of p21 or BTG2 alone are insufficient to fully predict the complex phenomena of cell cycle arrest and subsequent resumption of the cycle. This highlights the requirement of model extensions to predict the dynamics of temporary cell cycle arrests.

In contrast to DNA damaging agents that typically induce a cell cycle arrest, endocrine disrupting chemicals (EDCs) modulate cell cycle progression via hormone-like stimulation of such progression. To examine disruption of hormonal signaling, we study the effect of estrogen on the activation of the estrogen receptor alpha (ERα) and its downstream targets GREB1 and PR, and their role in the cell cycle (**Chapter 4**). We create an ODE model that combines the protein expression dynamics of ERα, GREB1 and PR with dynamics of cell cycle progression indicators CDK1 and APC/C. To evaluate the accuracy of our model-based predictions, we monitor cell cycle progression in single MCF7 breast cancer cells using live-cell microscopy. We develop a convolutional neural network for cell segmentation and create single cell tracks to quantify cell phase durations for individual cells. With our model, we simulate the effect of GREB1 and PR knockdowns on cell cycle progression and successfully predict the prolonged G1/S transition phase after GREB1 knockdown and the G1 arrest after PR knockdown. In conclusion, our model provides mechanistic insight in the regulation of cell cycle progression, and forms a solid basis for future research into the role of EDCs on cell cycle progression.

Because cell fate is not only determined by intracellular dynamics, but also by the microenvironment and cell-cell interactions, we create a spatial simulation model that simulates intra- and extracellular dynamics of hepatocytes and immune cells in a liver lobule after acetaminophen (paracetamol, APAP) exposure (**Chapter 5**). In this model, oxidative stress and consequent DNA damage inflicted by APAP metabolite NAPQI leads to necrotic cell death and senescence, the latter being determined by p21 expression. Surrounding immune cells induce the spread of senescence, yet are also responsible for liver recovery through the release of mitogenic biomolecules and clearance of senescent cells. We utilize this model to run *in silico* tests to investigate the efficacy of several therapies and the timing of their administration aiming to

neutralize effects of APAP overdosing. With this research, we incorporate intra- and extracellular sources that influence cell fate and therefore create a realistic computational liver model that can be used as backbone for modeling responses to alternative compounds.

Overall, in this thesis we present several computational models to simulate cellular responses to various compounds. In the process of model development and its application to experimental data, we identify open questions and generate new hypotheses on the mechanisms behind biological processes such as protein expression and cell fate. In addition, we show that computational modeling is an excellent tool to quantitatively integrate processes on different levels of biological organization. This specifically applies to our aim of unraveling intracellular protein dynamics upon chemical-induced stress and their quantitative relation to cell fate. Ultimately, such computational models can be used in quantitative adverse outcome pathways and thereby help to predict adverse reactions for novel compounds.