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Mathematical modeling of hematological malignancies

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1. ABSTRACT

Mathematical models addressing important aspects of hematological malignancies have recently facilitated an improved understanding of the involved complex biological processes and the prediction of potential targets for therapeutic approaches. These models investigate a wide spectrum of topics ranging from metabolic processes, gene regulatory networks and signal transduction up to the behavior of cell populations. However, despite this range of biological processes, the modeling strategies share many common features. Biological knowledge is translated into abstract descriptions representing complex networks and the parameters of these mathematical models are derived from literature data or estimated from experimental measurements. The established mathematical models are used to interrogate key properties of the investigated system by model simulations. These predictions are validated based on previously published or novel experiments. Additionally, new drug targets are predicted or novel insights into biological processes are provided. Here, we summarize the strategies employed to establish four mathematical models that address different processes in leukemia and lymphoma cells. Furthermore, we show how these systems biology approaches could contribute to elucidate the pathobiology of hematological malignancies.

2. INTRODUCTION

Mathematical oncology analyzes large datasets generated from imaging, proteomics and genomics studies and uses this information for the establishment of mathematical models. The investigation of biological processes by means of a mathematical model is the scope of the growing interdisciplinary field of systems biology. Systems biology combines quantitative data generation with mathematical modeling to discover common design principles that control emergent properties in complex networks (1).

The hematopoietic system is well-suited for mathematical modeling and experimental validation, since hematopoietic cells are readily extractable and can be quantitatively examined even *in vitro* (2). Furthermore, it has emerged that the molecular differences between both healthy and cancer cells as well as between different hematological malignancies are extremely complex. Mutations at various pathways and layers, feedback regulation and non-linear kinetics complicate the causal understanding and prediction of therapeutic targets. Additionally, the interaction of cancer cells with the immune system augments the complexity even further. Therefore, cancer was recognized as a „systems biology disease" (3).

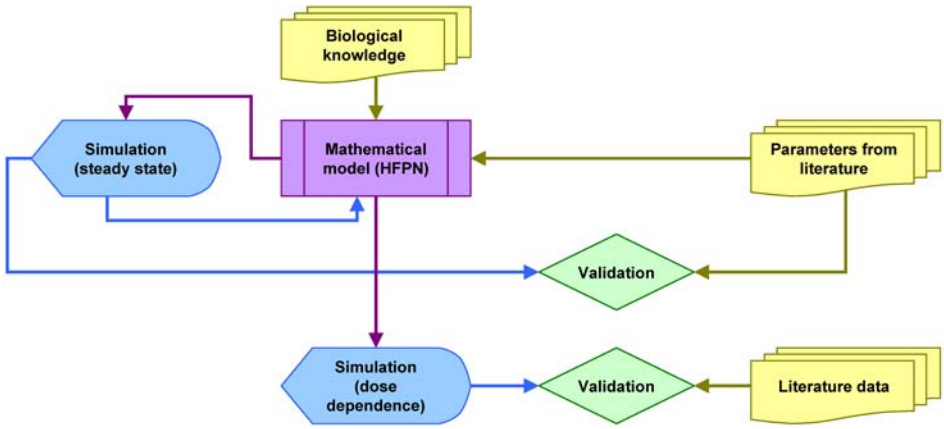

Figure 1. Drug metabolism model. Modeling strategy for a mathematical model predicting the effect of antifolate inhibition on folate metabolism (5). Literature information is depicted in yellow, models in purple, simulations in blue and validations in green. HFPN: hybrid functional petri net.

Here, we discuss different modeling approaches addressing specific questions in leukemia and lymphoma cells. We demonstrate that the modeling approaches employed common strategies to establish, parameterize and validate the models, although different mathematical and experimental methods were applied. Additionally, we discuss current bottlenecks in these techniques and perspectives for the future.

3. MODELING STRATEGIES APPLIED TO HEMATOLOGICAL MALIGNANCIES

3.1. Drug metabolism model

Antifolate drugs such as the antifolate methotrexate are widely used in the treatment of acute lymphoblastic leukemia and non-Hodgkin's lymphoma (4). Their mechanism of action is based on the inhibition of folate-dependent pathways that are involved in the biosynthesis of purines and pyrimidines. Therefore, the treatment with antifolates results in disruption of DNA synthesis and causes death of proliferating cells. To investigate the folate pathway under normal and antifolate inhibitory conditions, Assaraf *et al.* (5) have established a mathematical model based on hybrid functional petri nets (HFPN). HFPN (6) is a modeling approach that uses ordinary differential equations to represent transitions (events) that occur between places (conditions) of a Petri net graph. The modeling strategy of Assaraf *et al.* is schematically summarized in Figur e1. Biological knowledge of the folate metabolism was translated into ordinary differential equations based on Michaelis-Menten kinetics to establish a HFPN mathematical model. Additionally, the antifolate drugs were included as inhibitors of the respective reactions in the model. To calibrate the mathematical model, kinetic constants measured previously in the murine L1210 leukemia cell line (7) were incorporated. These parameters were used to simulate the steady-state concentrations of metabolic compounds whose concentrations were not known. Model validation was performed by comparing simulated concentrations of selected folates with experimentally determined concentrations at steady-state conditions. Subsequently, the model was employed to calculate the dose-dependent effects of different antifolates on the biosynthesis rates of purines and pyrimidines. These simulations, revealing distinct potencies of the inhibitors, could then again be cross-validated to literature data. In conclusion, the model allowed *in silico* evaluation of the inhibitory profiles of antifolates as an inexpensive and user-friendly alternative to cumbersome and slow dose-response experiments.

3.2. Population model

Chronic myelogenous leukemia (CML) represents a clonal disorder that is characterized by the Philadelphia chromosome, resulting in expression of the constitutively active protein tyrosine kinase BCR–ABL (8). The kinase inhibitor imatinib mesylate is currently the standard therapy for CML (9). To elucidate the dynamics of CML cancer cells including the response of the immune system, a mathematical model was developed by Moore and Li (10). Their modeling strategy is shown in Figure 2. Based on immunobiological knowledge, the mathematical model was established as a system of ordinary differential equations (ODE). The reactions describe the dynamics of naive T cells, effector T cells and cancer cells in the blood. Naive T cells are activated to differentiate into effector T cells, while cancer cells can be killed by the effector T cells. Furthermore, rates for recruitment, growth and death of the respective cell types were included. Model parameters such as the average number of cell death were taken from the literature. Other parameters were estimated from literature data such as half-life and population rate values. The resulting model was simplified using rescaling techniques to reduce the numbers of parameters. To determine critical parameters for the progression of the disease, simulations with varying parameters were performed. Latin hypercube sampling (11) was employed to randomly sample the parameter space in a well-distributed manner. By plotting the randomly chosen parameter values against the maximum number of cancer cells, it was demonstrated that only two parameters significantly determine the maximum number of cancer cells and thus the disease: the growth rate and the death rate

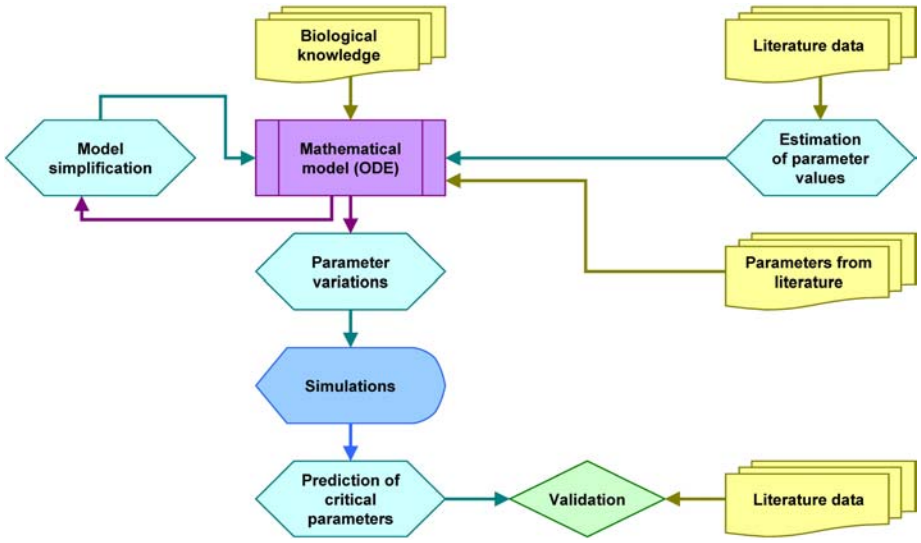

Figure 2. Population model. Modeling strategy for a mathematical model describing the dynamics of naive T cells, effector T cells and chronic myelogenous leukemia (CML) cells in the blood (10). Literature information is depicted in yellow, models in purple, computations in cyan, simulations in blue and validations in green. ODE: ordinary differential equation.

of CML cells. Of course, these are also the parameters that are affected by treatments against CML, as chemotherapy increases the death rate of cancer cells, while imatinib reduces the growth rate of these cells. Thus, the critical parameters predicted by the model are validated by literature data on CML treatment. Concluding, the model confirmed that treatment should focus on the cancer cells, rather than boosting the immune system.

3.3. Network model

T-cell large granular lymphocyte (T-LGL) leukemia is characterized by a clonal expansion of cytotoxic T lymphocytes (CTL) (12). Specifically, these cells have escaped activation-induced cells death (AICD) that normally limits the amount of antigen-primed T cells in the body. To understand long-term survival of competent CTL in T-LGL, Zhang *et al.* (13) established a Boolean network model of survival signaling. This modeling approach is summarized in Figure 3. To this aim, an extensive literature search was performed to construct a T-LGL survival signaling network. This network was simplified as much as possible while ensuring to maintain all causal relationships. To be able to perform simulations, this model had to be translated into a Boolean mathematical model. In a Boolean model, each node is described by two states (ON/OFF), while the regulation is represented by logic operators (OR/AND/NOT) (14). The resulting mathematical model allowed to perform simulations with different ligand inputs (IL-15, PDGF and antigen stimulation). The simulations revealed that constant stimulation with PDGF is required for long-term survival of leukemic T-LGL. This was validated experimentally by treating T-LGL leukemia peripheral blood mononuclear cells (PBMC) with a PDGF receptor inhibitor, specifically eliciting apoptosis. Furthermore, the model was used to predict deregulator proteins that determine the escape of CTL from AICD. This corresponds to the proteins in which a change of activity or amount will trigger apoptosis in T-LGL. As already nine deregulators were reported previously to be involved in inducing apoptosis in these cells, these deregulators were tested *in silico* by setting these nodes to the opposite state (e.g. ON to OFF) followed by analyzing the effect on apoptosis. As the model was in line with the literature data, this simulation was repeated with all nodes, resulting in a list of seven potential new deregulators, including SPHK1 and NF-kappaB. The relevance of the two latter proteins to apoptosis was then validated experimentally using PBMC from TGL-T leukemia patients and healthy donors. Thus, Boolean network modeling revealed novel deregulators determining the survival of CTL cells.

3.4. Dynamic pathway model

Classical Hodgkin's lymphoma (cHL) is a lymphoid malignancy characterized by the presence of malignant cells, which often account for not more than 1% of the tumor tissue, and by infiltrating cells of the immune system that constitute the rest of the cell mass. The malignant cells can be mononucleated (Hodgkin cells) and multinucleated (Reed-Sternberg cells) and in general are derived from mature, antigen-primed B cells (15). Primary mediastinal B-cell lymphoma (PMBL) is a locally highly aggressive non-Hodgkin lymphoma. Immunohistological studies provided evidence of a B-cell origin of PMBL (16). As both PMBL and cHL frequently share a constitutive activation of the JAK/STAT signaling pathway, Raia *et al.* (17) developed dynamic pathway models to predict common therapeutic targets. We depicted the modeling strategy of Raia *et al.* in Figure 4. The experiments were performed in two cancer cell lines representative of cHL and PMBL. The stoichiometry (i.e. the expression level) of signaling components has a large impact on signal transduction and is often altered in tumor cells. Therefore, the concentrations of signaling molecules were measured in the cell lines as well as in primary cells from healthy donors and major differences were detected. The

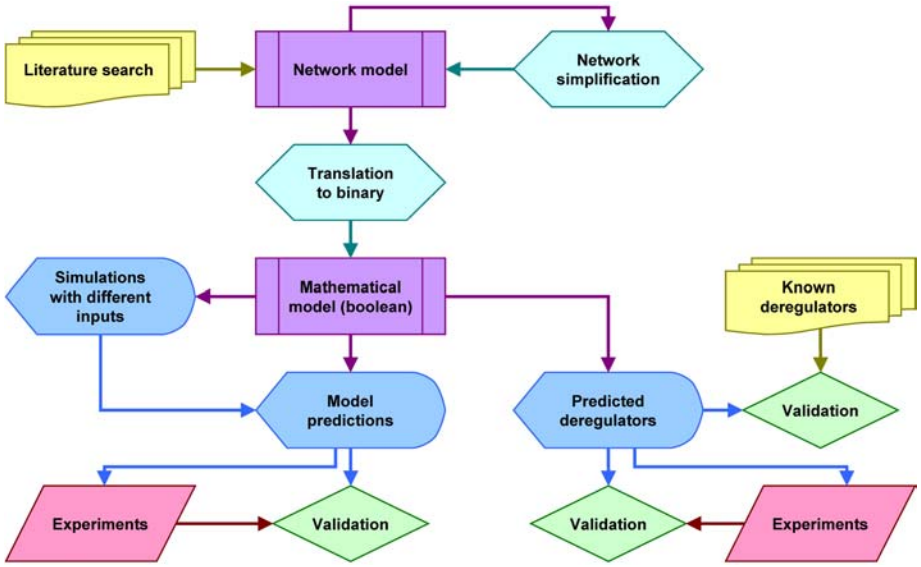

Figure 3. Network model. Modeling strategy for a mathematical model to predict signaling components determining the survival of cytotoxic T lymphocytes (CTL) in T cell large granular lymphocyte (T-LGL) leukemia (13). Literature information is depicted in yellow, models in purple, computations in cyan, simulations in blue, experiments in pink and validations in green.

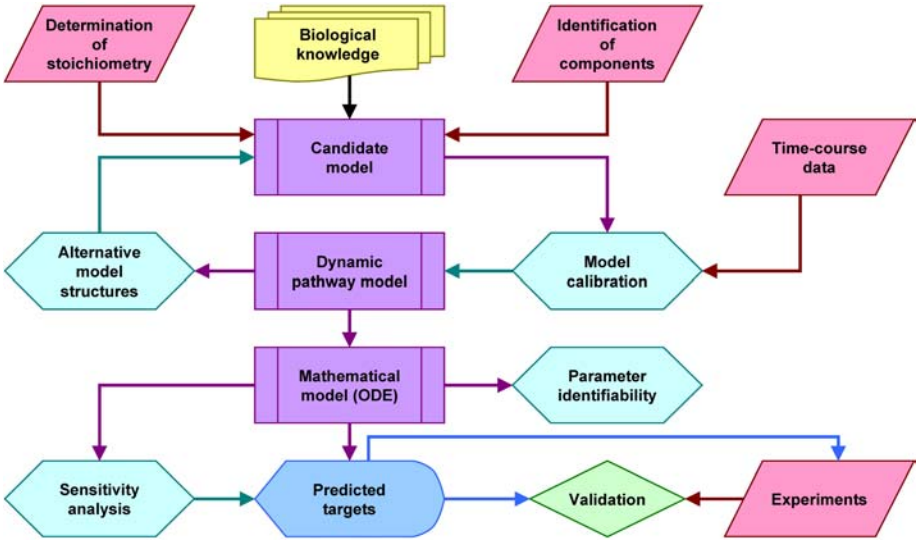

Figure 4. Dynamic pathway model. Modeling strategy for a mathematical model to predict therapeutic targets determining IL-13-induced signaling in Hodgkin and primary mediastinal B-cell lymphoma (17). Literature information is depicted in yellow, models in purple, computations in cyan, simulations in blue, experiments in pink and validations in green. ODE: ordinary differential equation.

stoichiometry was implemented into the mathematical models as initial concentrations of model variables. Established biological knowledge and novel experiments identifying the signaling components that are activated by IL-13 resulted in a ODE-based candidate model. Extensive time-course data with different doses of the ligand IL-13 were acquired by quantitative immunoblotting and used for parameter estimation. In case the candidate model could not describe the data, alternative model structures were tested until a dynamic pathway model for both malignancies was established that could explain all data sets. The resulting calibrated mathematical models were employed to perform a structural identifiability analysis. A parameter is structurally identifiable if it can be uniquely determined based on the model structure and the feasible measurements. As this was the case for all relevant parameters, the model could then be used to make quantitative predictions with a high confidence. A sensitivity analysis predicted the parameters that have the largest influence on a selected target gene, corresponding to potential drug targets. One of the predicted targets for both the cHL and PMBL model represented the phosphorylation

of STAT5, which was experimentally validated in the corresponding cell lines. In conclusion, dynamic pathway models were calibrated based on experimental data and employed to predict potential drug targets.

4. PERSPECTIVE

The mathematical models we discussed have used very different modeling approaches to answer various biomedical questions in leukemia and lymphoma cells. Despite these differences, surprisingly many similarities became apparent. The researchers all followed the cycle of hypothesis-driven research in systems biology as suggested by Kitano (18). This cycle begins with the review of biological knowledge and the selection of contradictory issues, followed by modeling and simulations, model predictions, experimental design and finally new experiments resulting in novel biological insights. Evidently, these approaches also have faced similar challenges. For example, one of the major bottlenecks is the lack of adequate data to calibrate and parameterize the mathematical models. While in some cases literature data on parameter values has been available and could be used for model calibration, more often parameters had to be estimated from experimental data. Furthermore, biological parameters such as rate constants of enzymes, stoichiometry of signaling components and the volume of cellular compartments can vary significantly between different cells. It is therefore essential to focus a particular model on a standardized cell system and experimental protocol (19). Additionally, if parameters are estimated from experimental data, these parameter can often not be determined uniquely, leading to non-identifiabilities that can affect model predictions (20). While recently powerful algorithms and modeling frameworks for parameter estimation have been developed (21), model establishment and selection is still a manual process requiring both profound knowledge of biological processes and comprehension of mathematical terms. Only in one of the publications discussed (17), alternative model structures were considered. Typically, mathematical models are established based on all available information, trusting that model validation will verify both the model structure and parameterization. While the experimental data for model calibration is difficult to obtain, acquiring data for model validation is surprisingly straightforward. The reason for this lies in the fact that once a mathematical model is established, it is relatively simple to simulate in the computer any experiment a researcher can think of. Similarly, if an experiment has been performed previously, it can be simulated using the model to validate the predicted results.

The different modeling strategies discussed here are characterized by distinct strengths and weaknesses. While mathematical models describing cell metabolism have proven to be successful in many cases, the complex regulation of enzymatic activities still poses a problem. For example, many glycolytic enzymes are subject to both allosteric and transcriptional regulation. Such detailed knowledge of the kinetic parameters is not necessary in Boolean network modeling. However, the results of this approach have to be considered with more caution, as Boolean modeling can only be performed by major simplifications of the actual biological processes. Dynamic pathway modeling on the other hand aims at describing the network's dynamics as accurately as possible. The need for accurate quantitative data as well as prior knowledge of the network topology represents the major bottlenecks of this approach. As cells never act in isolation, only a population model is able to adequately explain the course of a human disease. Evidently, population models require parameters describing cell type-specific responses and the cell-to-cell variability that are difficult to obtain. Therefore, modeling approaches have to be carefully balanced depending on the specific biomedical question and the kind and quality of data that can be obtained.

Concluding, while being still in its infancy, mathematical modeling of hematological malignancies has proven to be useful in tackling diverse biomedical questions. Current problems in hematology include stratifying patients in advance to optimize treatments as well as improving treatment regimes to prevent emergence of mutations, as seen for example in 20% of myeloma patients treated with imatinib (22). One way to overcome mutations rendering tumor cells resistant is the application of combinatorial treatments. Here, mathematical models of relevant pathways could contribute by identifying synergizing drug targets. Such combinatorial therapies could simultaneously prevent the emergence of drug-resistant tumor cells and improve drug safety by reducing side-effects. For patient stratification, mathematical modeling offers the chance to identify the mutations that are causative of the disease, rather than bystander mutations. Screens for these mutations would therefore enable oncologists to tailor treatments to the individual patient.

In the future, it will be important to not only model pathways and cells in isolation, but to establish multi-scale models linking various networks, time-scales and populations. This approach has been successfully applied to the human heart (23) and is currently in development for the human liver (24). A multi-scale model of human blood cancer encompassing cancer metabolomics, deregulated signaling networks and the interaction with the immune system would definitely offer new perspectives for both basic cancer research and translational medicine.

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