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MSM U01 Grant Number: EB023224

1. Project Title: Modeling Multiscale Control of Liver Regeneration

2. The aim of this collaborative U01 project is to develop a novel multiscale modeling framework that takes advantage of the in-depth information on cellular functional states provided by single cell data sets. We will develop the proposed multiscale modeling framework in the context of understanding the control principles governing liver regeneration. Our approach involves explicit accounting of cellular functional states of immune, stromal, endothelial and epithelial cells, and putative molecular processes driving the state transitions, with broad applicability to multiple tissue repair scenarios.

3. Details regarding the Model Credibility plan

A. List of Planned Actions

These actions are in alignment with the CPMS Ten Simple Rules, as listed in the below table.

B. Information gained by each Credibility Action

a. The single cell data contains information on the balance of cellular functional states, that can be used to tune the proportions of various cell phenotypes in the model
b. Spatial data provides tissue scale information on extent of liver sinusoidal formation, vasculogenesis, cell proliferation and location, etc. to tune the tissue scale model parameters
c. Not all parameters are expected to have equal influence over the liver regeneration outcome. Global sensitivity analysis will rank the parameters with highest influence over liver mass recovery, in the face of simultaneous variations in all the parameters, to help prioritize the experiments for estimating the corresponding parameters
d. Implementing multiple alternative models tests different assumptions

C. Actions and Activities classified within the CPMS TSR framework

#	Ten Simple Rules	Planned and Ongoing Actions
1	Define context clearly	Modeling molecular and cellular interaction network controlling liver regeneration response to injury. The organ level model of liver regeneration was designed to determine phenotypic shifts in hepatocytes that adversely affect liver regeneration. Specific details in the manuscripts.
2	Use appropriate data	The cellular network model was based on large scale transcriptomics and genome-wide transcription factor binding studies, wherein we identified the pathways and microRNAs that are differentially expressed due to chronic ethanol intake and/or partial hepatectomy. Additional data being collected includes single cell gene expression from multiple

		liver cell types, spatial data from intravital imaging, and noninvasive measures of liver growth and function. Other data from published literature on liver regeneration is utilized as relevant.
3	Evaluate within context	The models are being evaluated for match to physiological data from liver resection in normal and alcoholic liver disease models. All models are being evaluated using global sensitivity analysis. Parameters chosen for variation in the models are based on sensitivity analysis results and biological relevance.
4	List limitations explicitly	The limitations of each model were stated explicitly in the accompanying publications
5	User version control	Manual versioning for major changes to the model. Automatic version control is partly implemented using Dropbox for Business functionality to create a new version after each autosaving of the files.
6	Document adequately	Development process is not being documented prior to publication. However, institute-wide efforts are being made to introduce electronic laboratory notebooks that would improve model development documentation. The code is annotated to include relevant explanations to aid in understanding the model, when accompanied by the model equations and text from the associated manuscript.
7	Disseminate broadly	All model codes as well as code required to generate results shown in the manuscripts will continue to be provided as supplementary material with the respective publications. Model code on ModelDB is available for manuscript reviewers during peer-review. Model and data are disseminated via meetings. Manuscripts will continue to be published via open access.
8	Get independent reviews	So far, all the models were independently reproduced by laboratory colleagues not directly involved in the specific project. New members to the lab routinely review prior models as part of their initial training.
9	Test competing implementations	Organ-level liver regeneration model has been evaluated under competing implementations during independent review by laboratory colleagues, yielding similar results.
10	Conform to standards	Model development conformed to the typical practices followed in curated models published on the BioModels database. Models have been implemented in MATLAB and SBML formats.

D. Description of how the planned activities will lead to a credible model:
We are generating novel single cell transcriptomic data sets that will enable us to build multiscale models that account for temporal changes in cellular functional states during liver tissue response to injury, and relate these changes to overall tissue scale changes in physiological and metabolic functional aspects. By incorporating such data, and evaluating the model for critical parameters using global sensitivity analysis, and testing alternative assumptions for transitions between cellular functional states, we will be able to develop confidence on why certain network mechanisms are consistent with observed data and others are not. Open dissemination of the results, model code and associated data sets, will enable independent evaluation by others.

E. Progress to-date and Plans for next reporting cycle (6 months). What has been achieved since last reporting?
We developed an initial computational model that incorporates state transitions of macrophages, Hepatic Stellate Cells (HSCs), and hepatocytes, as well as the dynamics of key cytokines and growth factors, and JAK-STAT pathway kinetics in the hepatocytes. We tested the model predictions through isolation and transcriptional characterization of hundreds of single HSCs. Interestingly, our experimental results suggest four HSC transcriptional states as contributing to liver regeneration, three of which were represented in the model, and two of which are described for the first time in this work. Over the next project period, the model will be modified to incorporate the newly obtained experimental data on cell functional states.

Our independent review process has allowed us to test whether the documentation included in the manuscript is sufficient for a Modeling and Simulation practitioner, not necessarily familiar with the biological domain of liver regeneration, to reproduce the simulations. Based on this process of evaluation, we have improved our documentation and annotation approach in the manuscripts. For the next six months, we will continue to follow this productive approach.

4. Issues/concerns identified as critical or problematic to achieve the standard of credibility set by MSM Consortium
One issue is the lack of clear guidance on independent review. As there is no clear direction/consensus from the MSM Consortium, we have chosen to engage laboratory colleagues that are not otherwise involved in the project to start with the manuscript text, equations, parameter tables, and list of assumptions, to independently develop MATLAB or SBML code to attempt to reproduce the simulations and analyses. It would be helpful to obtain additional guidance on whether this sufficiently addresses the rule #8 in the above table.

MATLAB versus SBML implementations continue to be a source of concern, as we note that most users end up implementing the complex models in MATLAB even after they access the SBML version. So, we will limit the extent to which we will maintain parallel versions, and instead focus on MATLAB implementation at this time, with SBML versions produced if needed for publication according to corresponding journal requirements.

5. What other factors, if any, contribute to credibility but cannot be reported within the TSR structure?
None so far.