Modeling Multiscale Control of Liver Regeneration and Function

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We pursue a systems biology strategy that combines multiscale network modeling with the analysis of functional genomics and confocal microscopy-based data sets at the single cell scale to develop a mechanistic understanding of the factors that regulate liver function in health and disease.

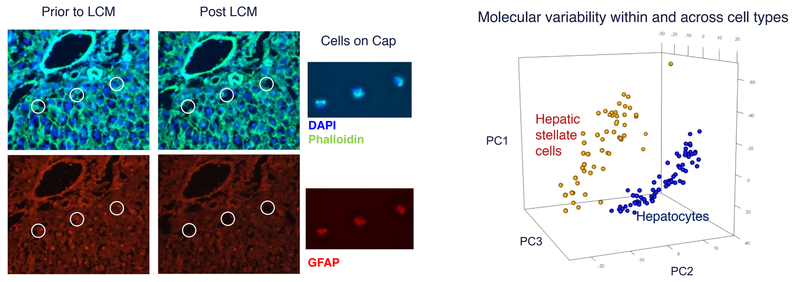
Single Cell Level Network Modeling[1]: We developed a computational model incorporating three signaling pathways with crosstalk (NF-κB, STAT3 and TGF-β) and two microRNAs (miR-146a, miR-21) that are differentially regulated by these pathways. This model was based on hypotheses derived from transcriptomics and genome-wide transcription factor binding studies (not shown). Our integrated model of signaling and microRNA regulation provides a new computational platform for investigating the mechanisms driving HSC molecular state phenotypes in normal and pathological liver physiology.

Spatial Modeling of Lobular Scale Response[2]: Ca^{2+} is a ubiquitous regulator of a wide variety of hepatocyte functions. In response to circulating stimuli, hepatocytes exhibit cytosolic Ca^{2+} spikes which propagate through hepatic lobules in a wave-like fashion. Such an organized Ca^{2+} response has been hypothesized to lead to a lobular scale coordination of downstream processes. We developed a receptor oriented, model of cytosolic Ca^{2+} spiking in hepatocytes and extended it to a lobular context by incorporating intercellular interactions. Our simulations predicted that spatial gradients of intracellular signaling components as well as intercellular interaction are required for lobular scale Ca^{2+} wave propagation.

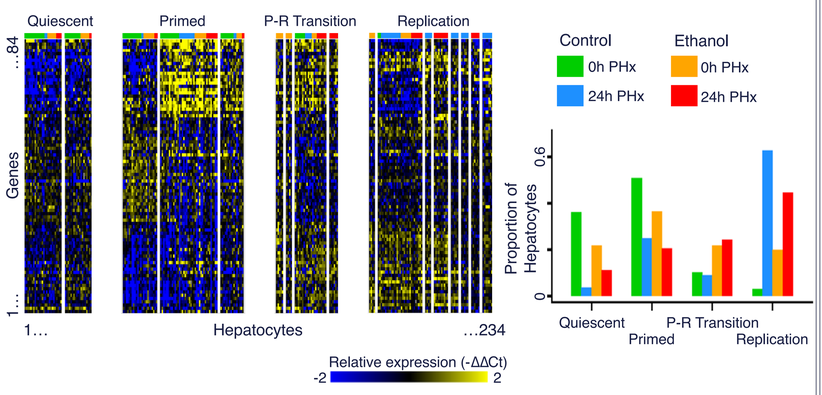
Organ Level Modeling of Liver Regeneration[3,4]: Chronic alcohol intake is detrimental for the regenerative response of liver. The impaired regeneration response may increase susceptibility to persistent liver damage after acute liver injury in alcohol-dependent individuals and thereby contribute to the onset of chronic liver disease. A better understanding of the mechanistic basis of this impairment has clinical implications for a wide range of ethanol-induced liver defects. We evaluated the tissue-scale consequence of the ethanol-mediated shift in the non-parenchymal cellular functional states using a novel computational model of the cellular and molecular networks driving liver regeneration following partial hepatectomy. Our modeling studies suggest that the ethanol-mediated disruption of dynamic state transitions of multiple cell types are necessary to yield defective regeneration. Consistent with our model predictions, our ongoing single cell gene expression studies suggest in hepatocytes as well as hepatic stellate cells show that chronic ethanol consumption shifts the distribution of hepatocytes and hepatic stellate cells (not shown) across a defined set of molecular states, with disruptive consequences on the overall liver tissue repair response following injury.

Transcriptomics and Confocal Microscopy

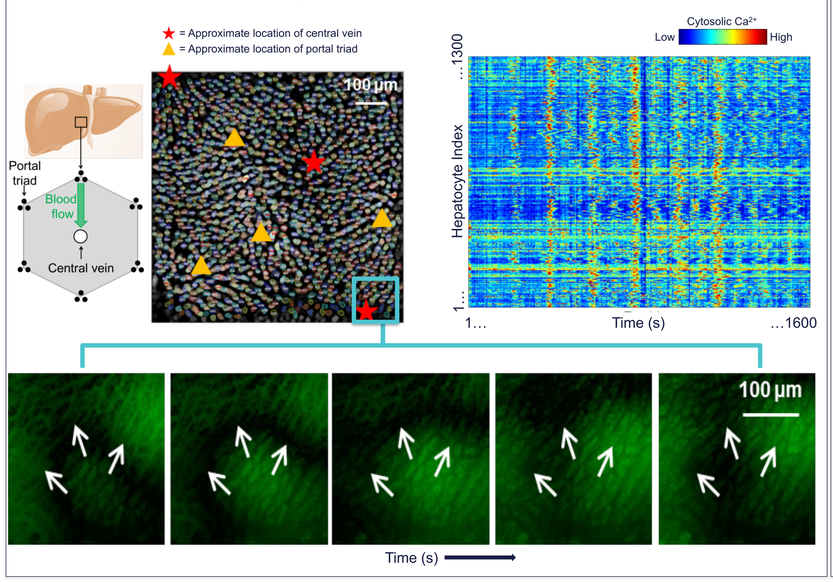
Laser capture microdissection (LCM) of individual hepatocytes and hepatic stellate cells enables high throughput and cell-type specific molecular profiling



Ethanol shifts the hepatocyte cell molecular state distribution away from a replicative phenotype in response to partial hepatectomy

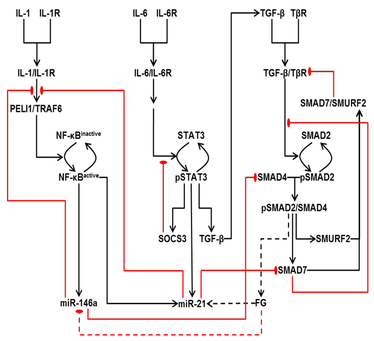


Sustained vasopressin stimulus induces spatially organized Ca^{2+} signal propagation in hepatic liver lobules

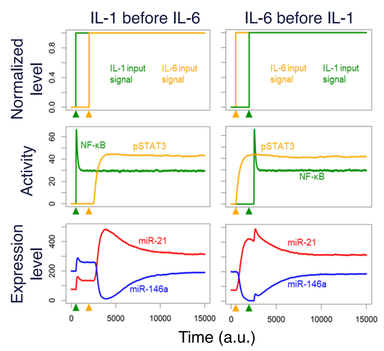


Single Cell Level Network Modeling

Network model of signaling and microRNA dynamics underlying hepatic stellate cell state activation

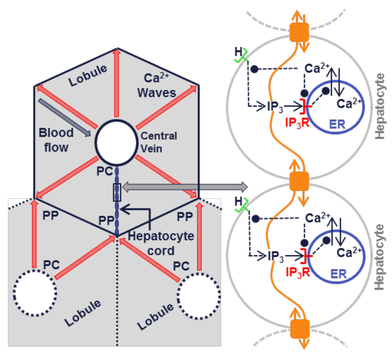


Relative timing of cytokine stimuli shapes microRNA expression dynamics in hepatic stellate cells

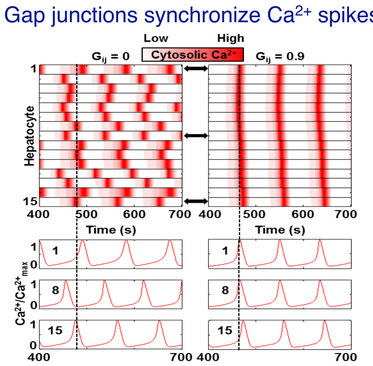


Spatial Modeling of Lobular Scale Response

1-dimensional model

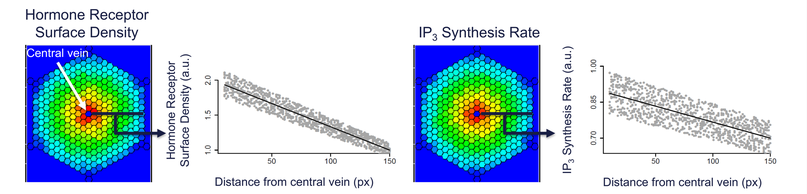


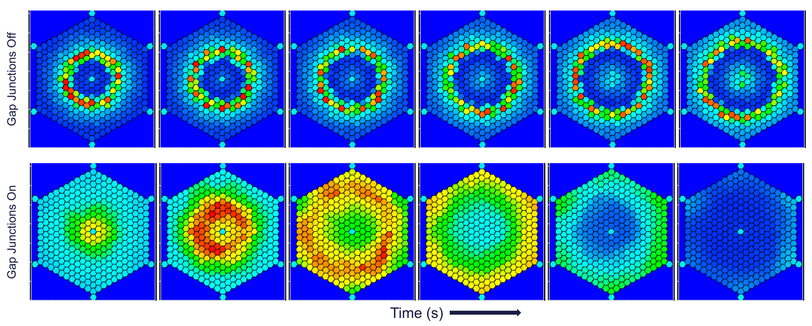
Gap junctions synchronize Ca^{2+} spikes



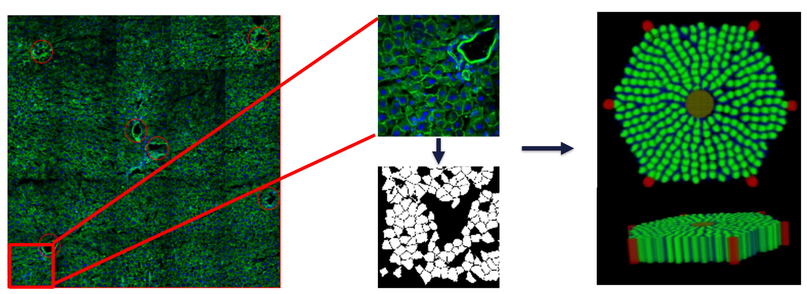
Two-dimensional model

Spatial gradients of intracellular signaling components and intercellular interactions are required for Ca^{2+} waves



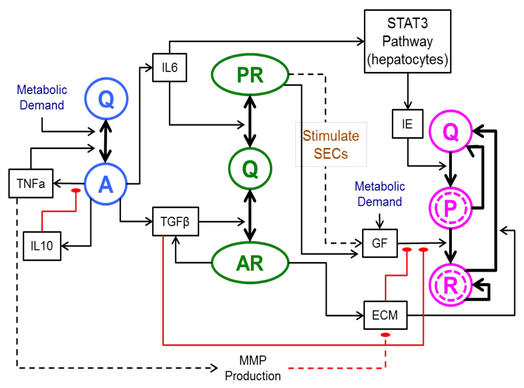


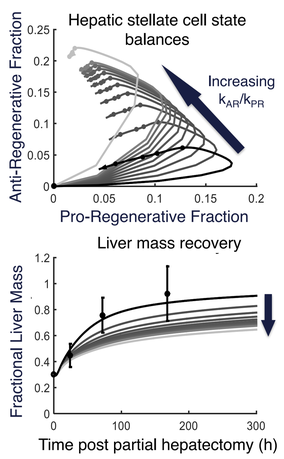
Towards three dimensions



Organ Level Modeling of Liver Regeneration

Imbalances in hepatic stellate cell state transitions impair the dynamics of regeneration





Credible Practice of Modeling and Simulation

Model Context	For the cellular network modeling, we sought to determine the relative contribution of NF-κB, STAT3 and TGF-β pathways, and miR-146a and miR-21 to the activation of hepatic stellate cells. In the lobular scale spatial model, we sought to identify the spatial patterns of heterogeneity in intracellular Ca^{2+} signaling capacity and cell-cell interactions that can lead to lobular scale Ca^{2+} waves observed experimentally. The organ level model of liver regeneration was designed to determine phenotypic shifts in hepatocytes that adversely affect liver regeneration.
Use of 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[5,6]. Spatial Ca^{2+} wave propagation model was based on previously published experimental studies as well as experimental data acquired by us wherein Ca^{2+} response in hepatocytes was elicited by vasopressin in isolated perfused mouse livers. Organ level model[4] of liver regeneration was built upon previously published models of liver regeneration[3,7].
Evaluation within context: Verification, Validation, Sensitivity Analysis and Uncertainty Quantification	The cellular network model and spatial Ca^{2+} wave propagation model were phenomenological, analyzing relative contributions of participating pathways/phenomena. A recent single cell RNA sequencing study provided evidence in support of the predictions from the spatial Ca^{2+} propagation model[8]. The predictions made by the organ-level regeneration model are currently being tested in ongoing experiments. All models were evaluated using global sensitivity analysis. Parameters chosen for variation in the models were based on sensitivity analysis results and biological relevance.
Model Limitations	The limitations of each model were stated explicitly in the accompanying publications[1,2,3].
Version Control	Automatic version control was not performed on the models.
Development Process Documentation	Development process was not documented prior to publication. However, institute-wide efforts are being made to introduce electronic laboratory notebooks that would improve model development documentation.
Model Dissemination	All model codes as well as code required to generate results shown in the papers were provided as supplementary material with the respective publications. A part of our work on spatial Ca^{2+} wave propagation was published in IEEE-TBME Special Issue on Model Reproducibility and was accompanied by the original Matlab code and an additional SBML implementation for cross-platform usability.
Independent Review	S far, all the models were independently reproduced by laboratory colleagues not directly involved in the specific project.
Competing Implementations	Spatial Ca^{2+} wave propagation model and organ-level liver regeneration model have been evaluated under competing implementations during independent review by laboratory colleagues, yielding identical results. Additional modified implementations yielded similar results, with differences that were informative of the impact of relaxing certain assumptions.
Conformation to Standards	Model developers conformed to the typical practices followed in curated models published on the BioModels database.
References for Data Sources and Model Dissemination	1: Kuttippurathu L, Parrish A, Vadigepalli R. Integrated computational model of intracellular signaling and microRNA regulation predicts the network balances and timing constraints critical to the hepatic stellate cell activation process. Processes. 2014 Oct 17;2(4):773-94. 2: Verma A, Makadia H, Hoek JB, Ogunnaike BA, Vadigepalli R. Computational Modeling of Spatiotemporal Ca^{2+} Signal Propagation Along Hepatocyte Cords. IEEE Transactions on Biomedical Engineering. 2016 Oct;63(10):2047-55. 3: Cook D, Ogunnaike BA, Vadigepalli R. Systems analysis of non-parenchymal cell modulation of liver repair across multiple regeneration modes. BMC systems biology. 2015 Dec;9(1):71. 4: Cook DJ, Achanta S, Hoek JB, Ogunnaike BA, Vadigepalli R. Cellular network modeling and single cell gene expression analysis reveal novel hepatic stellate cell phenotypes controlling liver regeneration dynamics. In review. 5: Dippold RP, Vadigepalli R, Gonye GE, Patra B, Hoek JB. Chronic ethanol feeding alters miRNA expression dynamics during liver regeneration. Alcoholism: Clinical and Experimental Research. 2013 Jan 1;37(s1). 6: Kuttippurathu L, Patra B, Hoek JB, Vadigepalli R. A novel comparative pattern count analysis reveals a chronic ethanol-induced dynamic shift in immediate early NF-κB genome-wide promoter binding during liver regeneration. Molecular BioSystems. 2016;12(3):1037-56. 7: Furchtgott LA, Chow CC, Periwal V. A model of liver regeneration. Biophysical journal. 2009 May 20;96(10):3926-35. 8: Halpern KB, Shenhav R, Matcovitch-Natan O, Tóth B, Lemze D, Golan M, Massasa EE, Baydatch S, Landen S, Moor AE, Brandis A. Single-cell spatial reconstruction reveals global division of labour in the mammalian liver. Nature. 2017 Feb;542(7641):352.

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