Application of Weighted Gene Co-Expression Network Analysis (WGCNA) to Dose Response Analysis.

Improving interpretation of nonclinical results using modularity to reduce complexity without loss of biological information.

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Multi-Scale Modeling of Pathophysiology

Liver Response
Liver Function Gate
Multicellular Signals
Cell Fate Gate
Cellular Response
Signal Integration
Signal Generation
Chemical
Organellar
Cell (HPC)
Multi-cell (HPC+NPC)
Organ/Tissue
Organism

Pathophysiological Complexity

Dynamic Complexity (dose/time)

Compound class specific

Tissue stereotypic

->adaptive/progressive->

HPC-hepatocellular
NPC-nonparenchymal

Liver fate -decision gate
HPC-hepatocellular
NPC-nonparenchymal

Multiscale/hybrid model

Δ initial conditions
Signal transduction
Signal integration

protein damage
autophagy
cell death
NPC activation
cholestasis; fibrosis

min-> hrs
hrs-> weeks
days->months

Multi-scale/hybrid model

k_{prolif}

normal

k_{inj}
damaged

k_{pop}
Overview

♦ Modeling biological complexity
  • The modular nature of complex systems.
  • Leveraging modular systems models using gene expression data.
  • Translating gene expression data into biological understanding.
  • Reducing redundancy in MSigDB.
  • Knowing what we don’t know.

♦ Understanding Molecular Pathogenesis
  • Correlating expression modules with pathology.
  • Closing the loop from transcription factor to pathogenesis.
  • Predicting adaptive vs progressive responses.
  • Closing the loop on transcriptional control (addendum slides).

♦ Applications of WGCNA to Dose Response Analysis
  • Separating injury signals from tissue stereotypic response.
  • Perturbing network in culture.
  • Translation to human.
Biological Systems are Modular Across Scales of Complexity

♦ Modularity refers to “…pattern[s] of connectedness in which elements of a system (e.g. mRNAs) are grouped into highly connected subsets.” (modified from Wagner et al.¹)
  • Modules can be arranged in hierarchies using looser connections between modules.

♦ Modular behavior can be captured in unsupervised network models using coalescent properties of the system.
  • Physical interactions – protein interaction networks
  • Dynamic interactions – gene regulatory networks
  • Statistical interactions – individual elements connected to phenotype

♦ Co-regulation in transcriptional networks is a coalescent property of biological systems – networks self-assemble.
  • Connected at level of transcriptional control, e.g. Hox gene networks, Nrf2, etc.
  • Defined/modelled statistically to yield co-expression modules.

♦ Modeling complex systems as networks/modules has advantages:
  • Avoids the ‘curse of dimensionality.’
  • If 2×10⁴ genes form 2×10² modules complexity is reduced by 99%.
  • Biological content is retained.
  • Network visualization applied to modular systems improves data interpretation.
WGCNA$^2$ - Form Follows Function

Co-expression modules (genes that respond similarly to drugs): 1 readout per module; the Eigengene (EiG).

WGCNA:85 - Atf4 nutrient depletion
Reducing Redundancy of MSigDB Information.

Screen shot from Spotfire TXG_MAP tool for WGCNA:Liver_8, a module highly enriched in genes associated with the proteasome.
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Predicting Adverse Responses for Adaptive vs. Progressive Tox-Phenotypes

Panels A and B: Average absolute eigengene score (Abs(aveEiG)) for all module changes for three dose levels of acetaminophen (A) and methylenedianiline (B) from TG-GATES 28d repeat dose rat liver studies. Note the scales for A and B differ. The TXG_MAP outputs shown correspond to the highest dose in the upper panel at different time points corresponding to the red circled time points.

PANELS C and D: Top-ranked modules selected for effect size >1.0 and p-adjust <10^{-3}. are shown for any tox-phenotypes considered adverse and either concurrent (C – present at the same time as gene expression) or predictive of adverse tox-phenotypes occurring at any time later than 1 day (D). Discussed in detail in Fig 2 of Sutherland et al. (2017).
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The Importance of Time Series

**BEST COMPARITORS:**
- bortezomib $r=0.7$
- cycloheximide $r=0.69$
- N,N diemethylnitrosamine $r=0.68$
- phorone $r=0.68$

**LATERAL:ISCHM:**
- Difference drivers relate to heat shock, cell cycle arrest, cell-cell junction changes

**CAUDAL:NON-ISCHM:**
- Difference drivers relate to ribosomal RNA processing
- Evidence of distinct responses

**lateral:ischemic @ 4 hr $r=0.51$**
- Lateral difference drivers relate to heat shock, cell cycle arrest, cell-cell junction changes
- Caudal difference drives relate to ribosomal RNA processing
- Evidence of distinct responses

**ISCHM:nonISCHM @ 1d $r=0.84$**
- Similarity driven by DNA replication, ribosomal biogenesis and tubulin formation
- Evidence of convergent pathobiology
Perturbations Caused by Placing Hepatocytes in Culture.

Average module score (degree of transcriptional perturbation)

A: mouse liver vs. TG rat liver
B: MPH vs mouse liver
C: 100 mg/kg methapyrilene @ 29 days
D: 30 mg/kg N-nitrosodiethylamine @ 15 days
E: HPH vs. human liver
F: 10 mg/kg cycloheximide @ 9 hrs
G: HepG2 vs. human liver
H: TG RPH vs. TG rat liver
I: DM RPH vs. DM rat liver
J: 2337 mg/kg aminosalicylic acid @ 1 day
K: 1 mg/kg bortezomib @ 9 hrs

**HYPOTHESIS:** Probability of Human Liver Toxicity Given Nonclinical Toxicity is a Function of Network EiG, Preservation and Effect Size.

\[
p(hLT|\text{rLT}) = f(\text{networks})
\]

\[
p(hLT|\text{rLT}) = f(\text{EiG, eff size, preservation})
\]

*Where:*

- **EiG** – eigengene score (How much did it change?)
- **Eff Size** – Is the network associated with adverse outcomes?
- **Preservation** - Z-score (Is the network preserved in human?)
QUESTIONS?
REFERENCES

