NETWORK METHODS FOR IDENTIFYING REGULATORS OF INFLUENZA A VIRUS INFECTION

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DRUG TARGET DEVELOPMENT IS NEEDED TO ADDRESS GLOBAL INFLUENZA INFECTION

Only three FDA-approved antiviral treatments available
- One is not recommended for children and people with breathing problems

**Question:** Can existing protein-protein interaction data be used to predict drug target candidates in a novel way?
TWO NETWORK APPROACHES TO DRUG TARGET DISCOVERY

1. Disease subnetwork
2. Cellular controllability

Influenza A Virus
Host Proteins
Virus Proteins
Influenza A proteins

Non-virus interacting host proteins

Virus interacting host proteins

**Degree:**
Number of interactions a protein is involved in

**Betweenness:**
Measure of network flow “bottleneckness”
PREVIOUS WORK USES NETWORK TOPOLOGY TO IDENTIFY DISEASE RELEVANT PROTEINS

Influenza proteins prefer to interact with proteins in significant network positions
Degree and betweenness p-values: <10^{-16}

Problem:
Topology is not sufficient as a guide for drug target discovery
Little analysis of downstream proteins
VIRUS-SPECIFIC SUBNETWORK METHOD FOR TARGET IDENTIFICATION

Connecting protein: Proteins between virus interacting proteins and proteins identified as relevant to virus replication in an siRNA screen

Analyze subnetwork proteins for potential as antiviral drug targets
SUBNETWORK POSITION ACTS AS PREDICTOR OF ANTIVIRAL DRUG TARGET CANDIDACY
SUBNETWORK PROTEINS ARE FUNCTIONALLY DISTINCT FROM VIRUS-INTERACTING PROTEINS

**Virus interacting:**
- Virus replication
- RNA transcription
- Protein translation

**Connecting:**
- Immune response
- NFkB pathway
Integrating virus-host interactions, siRNA data, and network topology methods can improve antiviral drug target discovery

The novel subnetwork method:
- Isolates disease specific pathways that allow for the promotion of viral replication
- Detects proteins that are traditionally unidentified by network methods
**Question:** How does the virus *manipulate* the cell to influence specific biological pathways?
To control a system, individual states must be driven to desired values.

Viral infection can be modeled as a controllability problem.
STEP 1: IDENTIFY MINIMUM CONTROL SET FOR CELLULAR CONTROL

After infection:
- Same proteins with 11 exceptions (Viral proteins)
- 8.9% of minimum control set also interact with viral proteins
- Significant betweenness compared to non-virus interacting minimum control proteins (p-value: $2.2 \times 10^{-16}$)

Minimum control set: 2

Infection does not alter **magnitude** of cellular control
STEP 2: OBSERVE CHANGES TO CONTROL USING DEPLETION ANALYSIS

Remove each protein to detect differences in minimum control set

Remove protein A:

Minimum control set: 3

Depletion analysis measures alterations in the ability to control the network
STEP 2: OBSERVE CHANGES TO CONTROL USING DEPLETION ANALYSIS

The absence of single proteins does not alter the control structure of the infected system.

Only changes seen are a result of the 11 changing minimum control proteins.

Fails to detect known changes in immune response and transcriptional processes.
STEP 3: IDENTIFY KEY PROTEINS USING GLOBAL ANALYSIS

Global analysis measures a protein’s significance to all ways a network can be controlled.
STEP 3: IDENTIFY KEY PROTEINS USING GLOBAL ANALYSIS

Global analysis identifies infection specific changes to network behavior

24 host proteins display a change in significance post-infection

All identified proteins are both minimum control and virus interacting proteins (2% of all proteins)
Network is more **difficult** to control in their absence

Often **globally significant** and involved in many ways to control the network

Responsible for the **ease** and **propagation of control** through the system
One possible drug target development strategy is to promote the protection of minimum control proteins.
Depletion method: no proteins with topological significance

Global method: high network significance during infection only
Protein set functions (IPA):

**Depletion** (11 changing minimum set):
mRNA processing (*CELF1, HNRNPA0, SF384, and SRPK2*, p-value: $3.33 \times 10^{-6}$)

**Global** (24 minimum set/virus interacting):
Protein synthesis, centered around **NF-kB**
Cell infection (*EPHA2, FBL, PFKM, PSMA5, SSR1, and TFRC*, p-value: $9.58 \times 10^{-4}$)

**Interferon regulated genes:**
Depletion: 11/11
Global: 20/24

6 Global proteins identified in >10 studies
Validation data from 6 partial siRNA screens for host factors involved in influenza replication

**Depletion:** 2/11 validated (fisher test p: 0.685)

SF3B4   SRPK2

**Global:** 5/24 validated (fisher test p: 0.252)

OSMR   PPA1   PSMA5   POLE4   GDI2

*Genes of interest may be outside of partial genome screens*

*What should be screened next?*
SUMMARY: CONTROLLABILITY

• A comparison of controllability analyses of healthy and infected cell networks reveals key regulators of cellular control

• 24 proteins are recommended for future drug target efforts based on:
  • Network characteristics
  • Controllability behavior
  • Biological relevance
THANK YOU

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