Systems biology

TROVE: a user-friendly tool for visualizing and analyzing cancer hallmarks in signaling networks

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Abstract

Summary: Cancer hallmarks, a concept that seeks to explain the complexity of cancer initiation and development, provide a new perspective of studying cancer signaling which could lead to a greater understanding of this complex disease. However, to the best of our knowledge, there is currently a lack of tools that support such hallmark-based study of the cancer signaling network, thereby impeding the gain of knowledge in this area. We present TROVE, an user-friendly software that facilitates hallmark annotation, visualization and analysis in cancer signaling networks. In particular, TROVE facilitates hallmark analysis specific to particular cancer types.


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1 Introduction

Cancer is a disease that is both complex and dynamic (Grizzi et al., 2006). The complexity of cancer stems from its heterogeneity and the intricacy of cell signaling within the biological system. For example, in triple negative breast cancer (TNBC) patients, key somatic genetic alterations found did not consistently apply across all patients and may depend on additional factors such as tumor morphology (Weisman et al., 2016). Recently, Hanahan and Weinberg proposed the notion of cancer hallmarks, which seeks to explain the complexity of cancer signaling (Hanahan et al., 2011). The 10 hallmarks comprises sustaining proliferative signaling, evading growth suppressors, avoiding immune destruction, enabling replicative immortality, tumor-promoting inflammation, activating invasion and metastasis, inducing angiogenesis, genome instability and mutation, resisting cell death and deregulating cellular energetics. These hallmarks can be mapped to various signaling pathways implicated in cancers (Li et al., 2014), and span the entire process of cancer initiation and development. Specifically, complexity of cancer signaling is embodied in the intertwining of various hallmarks (Floor et al., 2012).

Although cancer in general exhibit many or all of the ten hallmarks, the importance of individual hallmarks may vary from one type of cancer to another. For instance, activating invasion and metastasis and evading immune destruction hallmarks are highlighted as defining characteristics of TNBC (Dai et al., 2016). Similarly, the molecular species involved in each hallmark and the interactions between hallmarks may be different across different types of cancer. Hence, the study of cancer from the hallmark perspective has the potential to provide new insights to the understanding of this complex disease. However, to the best of our knowledge, there are no publicly-available tools that facilitate such study. In this paper, we present a user-friendly, graphical user interface (GUI)-driven software called TROVE (NeTwork-based hallmaRk annOtation, Visualization and characterEization) that supports hallmark annotation, visualization and analysis of cancer signaling networks.

2 TROVE

TROVE is implemented in Java and uses the PostgreSQL database for storing data such as hallmark annotations and the signaling networks. The current version of TROVE allows annotation, visualization and network topology-based characterization of cancer hallmarks for TNBC, estrogen receptor-positive (ER+) breast cancer and colorectal...
cancer. TROVE can be easily extended to support other cancer types by providing additional files (e.g. relevant GEO Omnibus dataset) and the exact steps are given in the user manual that can be downloaded along with TROVE.

2.1 Architecture
The TROVE architecture (Fig. 1, left) consists of the GUI and four modules, namely, cancer network construction module, network annotation module, network visualization module and hallmark characterization module. Given a user-specified cancer type (e.g. TNBC), the cancer network construction module generates the TNBC signaling network from the human signaling network (Cui et al., 2007) by extracting the induced subgraph of the set of genes implicated in TNBC. The human signaling network currently consists of 6305 nodes and 62906 edges. TROVE uses the entire gene set in GEO Omnibus repository that is relevant to TNBC (e.g. GSE38959) as reference for inclusion in the network. In order to minimize the likelihood of missing out genes in the TNBC signaling network, TROVE provides user with option to expand the generated cancer network with neighboring genes (up to three-hop) that are in the human signaling network, but not in the GEO dataset. Next, the network annotation module tags each node in the network with Entrez gene ID and hallmark annotations. The Entrez gene ID uniquely identifies each node in the network and is useful for resolving ambiguities in node identification. Hallmark annotation is performed by leveraging known mapping between gene ontology (GO) processes and hallmarks (Knijnenburg et al., 2015). The network visualization module handles the visualization of the annotated TNBC signaling network. Finally, the hallmark characterization module characterizes hallmarks based on topological features of nodes using support vector machine (SVM). TROVE uses a relational database as the backend to scale up to larger signaling networks. Note that TROVE allows users to add in additional cancer types for analysis through the GUI. Similarly, the input signaling network can be edited (e.g. add/remove node and edges) through the GUI. Details can be found in the TROVE user manual.

2.2 GUI-based hallmark annotation and visualization
The GUI of TROVE consists of four main panels (Fig. 1, middle). Panel 1 contains the list of nodes (molecular species) in the given network. Panel 2 provides a user with options for editing annotations (e.g. Entrez ID, hallmark, GO terms) of individual nodes. As it may not always be apparent which hallmarks best represent a particular node, TROVE provides a GO-assisted hallmark annotation feature to assist users on annotating (Expert users familiar with cancer hallmarks can choose to annotate nodes directly with relevant hallmarks.) the nodes. A list of GO terms is provided for selection and the hallmarks are assigned based on the GO-hallmark mapping. (Note that this mapping can be modified by the user via the GUI if required.). Panel 3 is a tabbed panel that displays details of the selected node including the signaling reactions it is associated with and its hallmark annotations. Panel 4 displays the annotated signaling network. Users are given the option of selecting the combination of hallmarks they would like to view. Nodes annotated with multiple selected hallmarks are displayed as multi-colored pie-charts (Fig. 1, middle), with each color representing one selected hallmark. A user can also visualize the network with respect to expression fold change and mutation frequency where the size of a node represents extent of change and different colors depict over and under expression.

TROVE supports the visualization of neighborhoods of nodes and pathways between nodes related to specific hallmarks. In particular, this allows users to answer questions such as what hallmarks dominate a particular pathway. For example, proliferation hallmark (annotated in green) features prominently in the neighborhood nodes of BRCA2 (Fig. 1, right). The presence of a dominating hallmark could indicate a significant contributory role of that pathway for the particular hallmark and nodes in these pathways could be further investigated as drug targets specific for the hallmark.

2.3 GUI-based hallmark characterization
In addition, TROVE provides functionality for hallmark characterization based on network topology. Briefly, hallmark characterization studies the relationship between topological features and hallmarks and identifies topological features that predict a particular hallmark. Hallmark characterization allows researchers to predict novel cancer hallmark genes based on defining topological characteristics and these novel genes may be targeted by combinatorial drugs hitting a variety of cancer hallmarks. The current version of TROVE supports 14 topological features (The topological features are in-, out- and total degree centrality; eigenvector centrality; betweenness centrality; bridging coefficient; bridging centrality; and undirected-, in-, out-, cycle- and middleman clustering coefficient.) and performs characterization using SVM following the approach in (Chua et al., 2015). Details can be found in the user manual.

3 Conclusions
TROVE facilitates the study of cancer signaling networks from the hallmark perspective by providing an integrated platform that allows construction of a specific cancer signaling network, assists
users in performing hallmark annotations, visualization and analysis of cancer hallmarks. An in-depth study of cancer signaling networks from the hallmark perspective can facilitate new design methodology for cancer drug combinations such as to select drugs that target cancer oncogenes in the same set or combinations of hallmarks.

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**References**


