Applications of p53 interactome analysis to personalized drug discovery

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Abstract.
The stress responsive transcription factor p53 is a powerful tumor suppressor implicated in over 50% of all human cancers. The complexity and nonlinear dynamics of p53 network coupled with extensive literature is challenging. Systems biology methodologies offer promising tools to investigate large networks, providing structured in silico representations for integrative analysis. The Boolean p53 interactome (PKT206) incorporates the diverse p53 information into a comprehensible framework, and demonstrates good predictive ratios (52–71%) using logical steady state analysis. Whilst extensive, Boolean models provide only a qualitative approximation of the system. A prerequisite of diseased in silico models is to accurately represent biological phenomena to characterize network perturbations for effective drug candidates. Thus a quantitative approach is necessary. We have applied a novel signal transduction score flow algorithm (STSFA) to PKT206 for model performance and comparison. The STSFA quantitatively analyses large scale ‘omics’ data, typically not accessible with large networks. STSFA in silico simulations resulted in accurate predictions when compared to various gene expression profiles (P = 1x10^-16), generating a significantly higher proportion of correct predictions than LSSA, (P = 0.003). Furthermore, genes CKS2, WWP1, EPHB4 were identified as prospective drug candidates for osteosarcoma cell treatment by in silico knockout analysis. In summary, refinement of Boolean PKT206 using STSFA has provided a semi quantitative view of the p53 interactome, and along with the use of ‘omics’ data, may be of greater clinical relevance for identification of perturbed pathways and personalized therapies by superimposition of individual genomic profiles.
1 Introduction

The stress responsive transcription factor p53 is a powerful tumor suppressor, and as such termed the guardian of the genome [1]. Since its discovery [2] the complexity of p53 functions has unraveled with more than 70,000 publications to date describing several hundred p53 responsive genes [3]. Its significance is highlighted with over 50% of all human tumors comprising p53 gene mutations or defects within its regulatory pathways [4]. In response to genotoxic and non-genotoxic stresses of DNA damage, hypoxia, and oncogenic activation [5], stabilized p53 transcriptionally regulates a plethora of downstream responsive genes via complex mechanisms [6], initiating anti-proliferative programs of cellular senescence, apoptosis and cell cycle arrest. The non-linear, synchronous dynamics displayed within the p53 network coupled with vast literature base makes it a challenging system to a greater understanding of p53 signaling pathways in tumor progression. Traditional reductionist methodologies fail to capture such disease dynamics [7]. A systems approach however, integrates diverse biological information into a coherent framework. Computational representations allow for amalgamation of molecular interactions into consistent depictions for in silico simulations to dissect biological systems. Biochemical networks of different mathematical complexities have been described which incorporate qualitative and quantitative kinetic and stochastic models [8, 9, 10], in an effort to represent dynamic biological systems. The Boolean p53 model designated PKT206 incorporates the extensive p53 signaling dynamics of DNA damage inducible pathways into a comprehensible network. The model considers 738 interactions of inhibition and activation between 206 nodes, with p53 as network hub, a DNA damage input of cellular stress, and outputs of cellular senescence and apoptosis [11]. Analyzed by logical steady state analysis (LSSA)[12], PKT206 predictions were validated with microarray profiles of human osteosarcoma U20S p53 +/+ and SaOS2 p53 -/- cells under DNA damaging conditions and HTC116 p53 wildtype and null colon cancer cell lines. Boolean models utilize directed graphs where nodes represent genes and edges their interactions [13]. Although informative, Boolean models are simplified and qualitative, providing only an approximate representation of the system [11, 12, 14]. Thus, these models are limited in their ability to truly depict biological phenomena. Higher predictive values are a prerequisite to accurately represent the system, to characterize network perturbations for effective drug candidates and clinical relevance [15]. Therefore, a quantitative approach is required to refine PKT206 towards a more robust model.

A novel signal transduction score flow algorithm (STFSA) has demonstrated good correlation with experimental transcriptome data and accurately predicts in silico multiple gene knockouts [16]. It quantitatively evaluates biological activities of a cyclic cellular network, and stimulates cell signaling flow on cyclic pathway topology. STFSA utilizes large scale ‘omics’ data to assign dynamic gene scores throughout the network from source nodes to final cellular processes for pathway analysis. Moreover, it incorporates crucial topological signaling information such as feedback cycles and protein node stoichiometry. As such, we have applied the STFSA to the PKT206 p53 interactome to enable quantitative analysis and improve model performance.
Analysis of predictions between both models was also undertaken using transcriptome data as previously described [11]. Furthermore, in silico knock out of anti-apoptotic hub nodes in osteosarcoma cell lines was investigated to identify candidate drug targets arising from pathway perturbations.

2 Material and methods

2.1 PKT206 p53 interactome and STFSA attributes

Original construction and analysis of the PKT206 p53 interactome is described in detail elsewhere [11]. STFSA utilizes transcriptome data (ChIP-Seq and/or microarray) for node score generation, and was provided as a Cytoscape plugin for versions above 2.7 [16]. Node and edge attributes were assigned to the network in accordance with [16], and imported into Cytoscape (v.3.0.2). For further investigation, gene expression profiles of HTCl16 p53 null and wildtype colon cancer cell lines were also analyzed from GSE10795 and [11, 17]. To overcome high background noise typically associated with microarray data [18], the median score of each gene in the network was taken as its expression value. Expression scores generated from microarray data were imported in a 3 tab-delimited .txt file, in accordance with [11]. However, as PKT206 comprises an extremely high number of nodes downstream to p53 (n=154), scores traversed to these were low and often negative. This would suggest that p53 has little influence on its downstream target nodes, when in reality it is the hub of the network. To overcome this, log₂ microarray scores were scaled up by a factor of 100. For HTCl16 data, expression scores were low, and as such raw microarray scores were additionally scaled up by the same factor. This allowed for an increased score in p53 for downstream signals, whilst leaving network ratios of edge and nodes unaffected.

2.2 Genome wide analysis of the predictive strength of the STFSA p53 interactome

To evaluate the predictive strength of the STFSA network on a genome wide level, in silico predictions of the score flow algorithm were generated and compared to experimental gene expression profiles. For simulation of the DNA damage effect, the DNA damage node was assigned an initial score, and node type attributes modified from ‘map’ to ‘gene’. For the control microarray data, a DNA damage node was added, using the initial experimental score of DNA damage. To ensure this value was neither too high or low, the mean of all other input log₂ expression values within control data was taken as the score. For in silico simulation of SaOS2 p53 -/-cells, the p53 node was deleted and the STFSA run using default U2OS untreated microarray data. Comparisons constructed between experimental and in silico simulations are described in Table 1.
Table 1. STFSA *in silico* simulations constructed from microarray profiles to test the predictive strength of the model against experimental data

<table>
<thead>
<tr>
<th>Microarray data</th>
<th>Experiment source</th>
<th>Experiment target</th>
<th>Simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SaOS2, p53 */- and U2OS, p53 */+</td>
<td>U2OS under DNA damage</td>
<td>SaOS2 under DNA damage</td>
<td>p53 wt DNA damage on vs p53 null DNA damage on</td>
</tr>
<tr>
<td></td>
<td>U2OS no DNA damage</td>
<td>SaOS2 no DNA damage</td>
<td>p53 wt DNA damage off vs p53 null DNA damage off</td>
</tr>
<tr>
<td></td>
<td>U2OS no DNA damage</td>
<td>U2OS under DNA damage</td>
<td>p53 wt DNA damage off vs p53 wt DNA damage on</td>
</tr>
<tr>
<td></td>
<td>SaOS2 no DNA damage</td>
<td>SaOS2 under DNA damage</td>
<td>p53 null DNA damage off vs p53 null DNA damage on</td>
</tr>
<tr>
<td></td>
<td>U2OS no DNA damage</td>
<td>SaOS2 under DNA damage</td>
<td>p53 wt DNA damage off vs p53 null DNA damage on</td>
</tr>
<tr>
<td></td>
<td>U2OS under DNA damage</td>
<td>SaOS2 no DNA damage</td>
<td>p53 wt DNA damage on vs p53 null DNA damage off</td>
</tr>
<tr>
<td></td>
<td>HTC116 p53 */- and p53 */+ no DNA damage</td>
<td>HTC116 p53 */- no DNA damage</td>
<td>p53 wt DNA damage off vs p53 null DNA damage off</td>
</tr>
</tbody>
</table>

STFSA *in silico* simulations were performed as in LSSA using the same omics profiles to mimic experimental conditions of gene activity within the network. Comparison of both algorithms were also analysed. wt = wild type. p53- = knockout.

2.3 STSFA score fold change analysis

The log₁₀ fold change (FC) in the STSFA scores between experimental and simulated data was calculated for each node (n=204), defined by the equation:

\[
\text{FC}(i) = \frac{\text{M1}(i)}{\text{M2}(i)}
\]

(1)

Where M1(i) is the median of expression values in the target condition and, M2 (i) the median of expression values in the source condition. Threshold values, (θ) were applied to normalize expression profile distributions [19], using mean value (x) and standard deviation (σ) of the log₁₀ FC scores, of (θ_{max} and θ_{min}), defined as:

\[
\theta_{\text{max}} = x + \sigma
\]

\[
\theta_{\text{min}} = x - \sigma.
\]

(2)
Gene activity was further determined, where:

\[
\log_{10} \text{FC} > x + \sigma \text{ means upregulated}
\]

\[
\log_{10} \text{FC} < x - \sigma \text{ means downregulated}
\]

\[-\sigma \leq \log_{10} \text{FC} \leq +\sigma \text{ means no change}\]

(3)

For comparison of both datasets and model evaluation, changes in each gene response between experimental and simulated conditions were analyzed using a similar approach previously described [20]. Predicted changes of gene activity may be described by a variable \(E_{\text{mod}}\) of 3 states (upregulated, downregulated, no change), differences between experimental and predicted simulations were defined as \((E_{\text{mod}} - E_{\text{exp}})\), where a correct prediction was defined as experimental and simulated outcomes both the same, a small error prediction being one outcome of 'no change' and the other 'up or downregulated' and a large error prediction as one outcome 'upregulated' and the other 'downregulated'. Frequency distribution between experimental and simulated data, and significance of comparison between LSSA and STSFA (Unpaired T Test) were undertaken using GraphPad Prism 6.

2.4 Determination of incorrect genes

For each condition (Table 1), any genes classified as ‘small error prediction’ or ‘large error prediction’ were ranked based upon the number of times a response was incorrectly predicted. A small or large error was assigned a score of 1, or 2 respectively. For example, a large error prediction in three conditions, and a small error in one condition, would result in a total score of 7. A threshold of 4 was defined for the response of a gene being consistently incorrectly predicted. To achieve this, a gene must receive a small error prediction in at least 4 simulations, or a large error more than once, or a large error and 2 small error predictions. This indicates that effects of experimental conditions on gene activity are incorrectly predicted within the network.

2.5 In silico knock out analysis of anti-apoptotic nodes

Potential therapeutic target was defined as a gene whose deletion from the network would result in a significant increase in apoptosis activity score, with minimal effects on the rest of the network. Knock out simulations of potent anti-apoptotic genes was performed by their in silico deletion and compared against default U20S untreated gene expression dataset for analysis of network perturbations.
3 Results

To validate the STSFA network, the log_{10} FC in gene expression scores, given in material and methods section, equation (1), between experimental and in silico conditions for each simulation (Table 1), were calculated and defined by the thresholds given in equation (2) for each comparison (Fig. 1). The STFSA model accurately predicted gene activity changes between both conditions of DNA damage and p53 knockout tests simulations for the majority of genes, with correct predictions made for 73 to 77% of the genes (P≤1x10^{-16}) (Table 2). Considering this accuracy, comparison against the Boolean model performance and prediction was further evaluated. Distributions of correct, small error and large error predictions were compared across all conditions (Fig. 2A). The STFSA consistently demonstrated a higher mean proportion (76%) of correct predictions when compared to LSSA (61%) in U20S p53 +/- and SaOS2 p53 +/- simulations (P < 0.005) (Fig. 2B). Similar results were observed for HTC116 p53 wild-type and null cell lines in the absence of DNA damage. For every condition, the proportion of correct predictions was significantly higher than would be expected if random data were used (P < 1x10^{-16}). Taken together, these results demonstrate the STSFA can accurately predict gene activity changes between transcriptome data and in silico simulations. Moreover, the STFSA model is consistently more accurate than the logical p53 model providing a semi quantitative representation of PKT206.

Table 2. Distribution of all predictions made by the STFSA p53 model for each in silico simulation compared to microarray data (percentage numbers rounded to the nearest whole number)

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Prediction</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>p53 wt DNA damage off vs p53 wt DNA damage on</td>
<td>True</td>
<td>159</td>
<td>44</td>
<td>4</td>
</tr>
<tr>
<td>p53 wt DNA damage on vs p53 null DNA damage on</td>
<td>True</td>
<td>158</td>
<td>47</td>
<td>2</td>
</tr>
<tr>
<td>p53 wt DNA damage off vs p53 null DNA damage off</td>
<td>True</td>
<td>158</td>
<td>43</td>
<td>6</td>
</tr>
<tr>
<td>p53 wt DNA damage off vs p53 null DNA damage on</td>
<td>True</td>
<td>152</td>
<td>52</td>
<td>3</td>
</tr>
<tr>
<td>p53 null DNA damage on vs p53 null DNA damage on</td>
<td>True</td>
<td>156</td>
<td>49</td>
<td>2</td>
</tr>
<tr>
<td>p53 wt DNA damage on vs p53 null DNA damage off</td>
<td>True</td>
<td>170</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>HTC116 p53 wt DNA damage off vs p53 null DNA damage off</td>
<td>True</td>
<td>137</td>
<td>32</td>
<td>3</td>
</tr>
</tbody>
</table>

Model predictive performance was evaluated with various expression profiles. Gene activity changes between experimental and simulated conditions were analyzed using the approach given in (3).
Fig. 1. Frequency distribution graphs of the log$_{10}$ fold change in STSFA gene activity scores, for each comparison between experimental and simulated conditions. Two thresholds are shown for each condition, $x - \sigma$ (blue bars) and $x + \sigma$ (red bars) given in (1). Genes whose log$_{10}$ FC of gene activity score fell between these were classified as ‘no change’, those above and below threshold values were ‘upregulated’ or ‘downregulated’, respectively. A) p53 null DNA damage on vs p53 null DNA damage off. B) p53 wt DNA damage off vs p53 null DNA damage off. C) p53 wt DNA damage on vs p53 null DNA damage on. D) p53 wt DNA damage on vs p53 wt DNA damage off. Other data not shown.
Fig. 2. The STSFA p53 network model is able to correctly predict the response to DNA damage and p53 knockout and is more accurate than LSSA. 

A) The distribution of all predictions made in each simulation between STFSAs and LSSA, defined by the $x \pm \sigma$ threshold. (DD) DNA damage.

B) Comparison of mean percentage of correct predictions for each PKT206 SaOS2 p53 $^+$ and U2OS p53 $^-$ simulations from Table.2 (Unpaired T Test, $P = <0.005$), ($x \pm$ SEM).
3.1 In silico knock out analysis of anti-apoptotic genes

Given the high level of predictive accuracy demonstrated by STFSA p53 simulations, in silico knock out (KO) analysis of anti-apoptotic genes was carried out to identify potential novel therapeutic targets (Fig.3). Apoptosis is well studied and is the most clinically relevant anti-proliferative program. Thus identification of apoptotic drug candidates is pivotal to effective cancer therapies. Genes that affected only the apoptotic pathway with little effect on the rest of the network were chosen: CKS2, IER3, C12orf5, WWP1, PSEN1, EPHB4 and PRSS50. CKS2 (essential for cyclin dependent kinase function), WWP1 (E3 ubiquitin ligase, regulates several tumor associated proteins) and EPHB4 (angiogenesis factor) were considered as potential chemotherapeutic targets [21, 22, 23]. KO analysis in the various target node deletion backgrounds demonstrated increased apoptotic log$_{10}$ FC scores compared to wildtype background. Little effect on the rest of the network was observed in each simulation indicative of them as potential candidates for drug development in human osteosarcomas.

![Gene KO Log$_{10}$ fold change](image)

**Fig. 3.** Log$_{10}$ fold change of STSFA activity scores for apoptosis and the $x + \sigma$ threshold for each in silico gene knockout. Increased apoptotic scores were observed in target gene deletion backgrounds.

4 Discussion

Here we have applied a novel algorithm [16] to the recently described PKT206 p53 interactome. We have investigated STFSA predictive strength using raw expression data from U2OS p53 +/+ and SaOS2 p53 -/-, and HTC116 colon cancer p53 null and wildtype cell lines [11]. Finally, we have compared STFSA to LSSA for model performance.
Dynamic biological phenomena such as cancer display considerable system complexity and heterogeneity. Multivariate dysregulation of signaling pathways governing fundamental cellular processes are typical to such processes [24, 25]. Thus, in silico representations must encode mathematical models of a quantitative nature for true representation [26]. The STFSA p53 interactome described here has accurately predicted overall p53 system attributes in response to DNA damage inducible pathways when compared to gene expression data. These results provide a semi quantitative representation of the p53 system as opposed to the qualitative Boolean approach. Furthermore, to investigate potent anti-apoptotic candidates, we simulated in vivo mutations of target nodes in various in silico knock out backgrounds. Here we describe WWP1, CKS2 and EPHB4 as putative cancer drug targets in human osteosarcomas.

Greater predictive ratios were obtained utilizing STFSA than LSSA, achieved by incorporation of quantitative processes during signal flow pathway analysis not considered in LSSA. Deterministic qualitative models such as Boolean often fail to capture the heterogeneity and stochasticity associated with large ‘omics’ driven biochemical networks. Boolean logic assigns static gene states only, of (“on/1”) and (“off/0”) corresponding to expressed or non-expressed respectively, thus are limited for true system representation. Discrete models provide good approximation of the qualitative behavior of the system [12, 14]. These networks may be represented by directed graphs where transfer states of edges are derived using logical operators AND, OR and NOT [13]. In naïve Boolean models no reaction rates are incorporated in transfer functions, thus no kinetic parameters are required. This simplicity allows for large scale modelling, and has been applied successfully to cancer networks [27, 28, 29]. However, Boolean models have limitations. Differential gene expression exhibits considerable inherent stochasticity and ambiguity, thus cannot be described sufficiently by two states. Poor predictive accuracy may be a consequence of the inference of deterministic functions. Indeed, many simplistic approaches appear robust when analyzed in silico, however display considerable reduced enactment once applied to experimental ‘omics’ data and, can only be applied for approximates and statistical system analysis rather than experimental representation [30,31].

The STFSA captures gene dynamics, of an empirical approach utilizing ‘omics’ data driven gene enrichment of a particular pathway. It incorporates stoichiometric processes as score partitions, stimulating cellular signal flow on cyclic pathway topology rather than static node states of steady state expression models. Even so, limitations of STFSA were observed since there remain incorrectly predicted genes. Those identified were in the majority downstream and connected only to p53. Considering the plethora of upstream genes influencing p53 activity this is not surprising, with any small node error traversing to p53. One factor to consider is that p53 has several isoforms differing at the N and C termini arising from the presence of a second promoter in intron one, alternative mRNA splicing and translation initiation, due to numerous post translational modifications [32, 33, 34]. The PKT206 p53 node represents all gene isoforms. Different isoforms have distinct roles and binding partners,
thus amalgamation into one node may compromise its ability to simulate the response of signaling pathways to changes in experimental conditions. Consideration of integration of these isoforms may resolve such network limitations. Nevertheless, the STSFA model described here provides a more robust and semi quantitative representation of the p53 interactome. This offers a promising platform for a fully quantitative, predictive transcriptional p53 model. Moreover, with the availability of high throughput methodologies, use of ‘omics’ data with STFSA p53 pathway analysis provides the next step for superimposition of individual expression data. This may be of greater clinical relevance for personalized targeted cancer therapy in p53 wildtype and negative tumors.

References