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Identifying the critical state of complex biological systems by the directed-network rank score method

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Abstract

Motivation: Catastrophic transitions are ubiquitous in the dynamic progression of complex biological systems; that is, a critical transition at which complex systems suddenly shift from one stable state to another occurs. Identifying such a critical point or tipping point is essential for revealing the underlying mechanism of complex biological systems. However, it is difficult to identify the tipping point since few significant differences in the critical state are detected in terms of traditional static measurements.

Results: In this study, by exploring the dynamic changes in gene cooperative effects between the before-transition and critical states, we presented a model-free approach, the directed-network rank score (DNRS), to detect the early-warning signal of critical transition in complex biological systems. The proposed method is applicable to both bulk and single-cell RNA-sequencing (scRNA-seq) data. This computational method was validated by the successful identification of the critical or pre-transition state for both simulated and six real datasets, including three scRNA-seq datasets of embryonic development and three tumor datasets. In addition, the functional and pathway enrichment analyses suggested that the corresponding DNRS signaling biomarkers were involved in key biological processes.

Availability and implementation: The source code is freely available at https://github.com/zhongjiayuan/DNRS.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Many complex systems undergo a critical transition and then switch abruptly to a contrasting state (Scheffer *et al.*, 2009); that is, there exists a so-called tipping point at which a drastic, irreversible and qualitative transition may occur. Detecting such tipping points for general systems, such as socioecological (Pananos *et al.*, 2017), financial (Billio *et al.*, 2016) and climate systems (Boers, 2018), has received extensive attention. In biomedical fields, a similar critical state transition is also observed in a variety of biological processes. For example, some chronic diseases may experience a gradual process that takes several years or even decades before catastrophic deterioration occurs. Cell fate commitment is also regarded as a critical transition of embryonic development, after which there is a drastic change in the cell populations (Zhong *et al.*, 2021). Identifying such a critical transition or tipping point in biological systems plays an essential role in providing insights into the underlying mechanism of disease progression or embryonic development (Guo *et al.*, 2021a; Shi *et al.*, 2021). The time evolution of a complex biological system is usually modeled as a time-dependent nonlinear dynamic system (Liu *et al.*, 2020, 2021a), in which the abrupt transition is viewed as the phase shift at a bifurcation point (Scheffer *et al.*, 2009). Therefore, from a dynamic system perspective, a biological process can be broadly divided into three states or stages (Fig. 1A): (i) a before-transition state, a stable state with strong resilience; (ii) a critical/pre-transition state, which is an unstable state just before the onset of qualitative changes and with low resilience and sensitivity to perturbation; and (iii) an after-transition state, another stable state with strong resilience after the qualitative transition. For biological systems, hunting for such a pre-transition state may provide appropriate timing for intervention to prevent or at least prepare for the upcoming catastrophic consequences, such as disease onset or deterioration. The recently proposed applications of a new concept, i.e. the dynamic

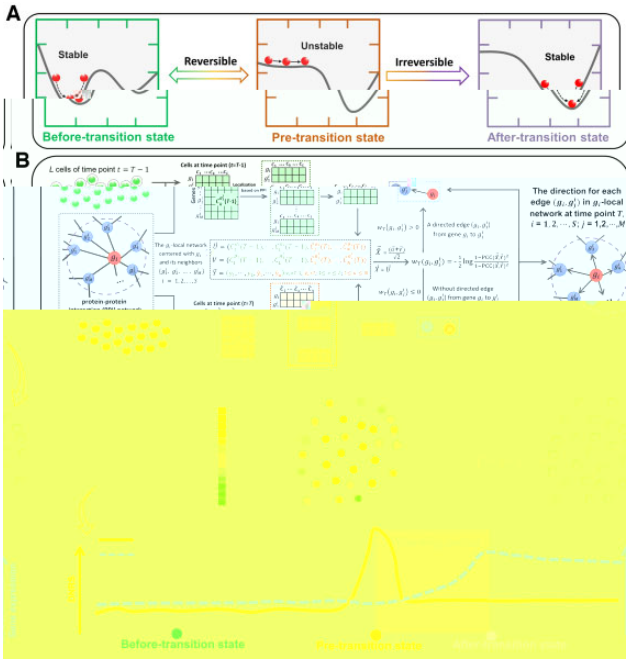


Fig. 1. Illustration of the proposed method for detecting the critical transition of complex biological systems based on a modified personalized PageRank. (**A**) The complex biological processes can be roughly divided into three stages/stages, i.e. a before-transition state, a pre-transition state and an after-transition state. (**B**) Given a group of control cells/samples from former time point t = T − 1 and a set of case cells/samples derived at time point T, the time-specific directed network of time point T is constructed based on rewiring the protein–protein interaction (PPI) network with direction determination index w_T. (**C**) The local DNRS is calculated for each node in the time-specific directed network of the time point T based on the personalized PageRank (Eq. 2) and then the DNRS (Eq. 8) is utilized to detect the early-warning signal for the critical transition of a complex biological system. (**D**) During the dynamic progression of a complex biological system, the DNRS remains low when the system is in a before-transition state, while it increases significantly when the system is close to the pre-transition state. Such an abrupt increase in the DNRS indicates the tipping point (or the critical state) of a complex biological system

network biomarker (DNB) (Chen *et al.*, 2012), have been employed to detect the critical transitions in biological systems. Specifically, when the system approaches the tipping point/critical state, a dominant group of strongly fluctuating variables (DNBs) appears and exhibits strong cooperative effects on molecular associations, which could be employed to quantitatively characterize the stability and criticality of a system at the network level (Chen *et al.*, 2022; Guo *et al.*, 2018; Liu *et al.*, 2021b). In the past, many network-based ranking techniques, such as random walk-based methods (Roy *et al.*, 2014; Winter *et al.*, 2012), topological property-based approaches (George *et al.*, 2006; Krauthammer *et al.*, 2004), the Markov random field model (Ma *et al.*, 2007) and the order statistical index (Aerts *et al.*, 2006), have been proposed for the identification of network-based disease biomarkers by exploring the cooperative effects of gene combinations. However, these studies mainly focused on the diagnosis of disease (static biomarker) instead of disease prediction (dynamic biomarker).

Recently, network-based methods from the DNB framework have been developed to address different biological topics, such as the identification of pre-disease states for complex diseases (Liu *et al.*, 2022; Peng *et al.*, 2022), the personalized characterization of diseases (Liu *et al.*, 2017; Zhang *et al.*, 2021) and the discovery of personalized driver genes prioritization (Guo *et al.*, 2021b). From the perspective of gene associative and cooperative effects, we presented a model-free computational method in this study, i.e. the directed-network rank score (DNRS) method, to detect the critical states of complex biological systems and identify key genes involved in related essential biological processes. Specifically, based on the cells/samples from former time point $t = T - 1$, the time-specific directed network at a time point T is constructed based on rewiring the protein–protein interaction (PPI) network with direction determination index $_T$ (Fig. 1B), and then the local DNRS is calculated for each node/gene by using the proposed personalized PageRank (Fig. 1C). The DNRS can be utilized to quantify the dynamic changes in gene cooperative effects of a time-specific directed network. The drastic increase in such DNRS signals an upcoming tipping point or pre-transition state of a biological system (Fig. 1D). Furthermore, at the identified tipping point, a group of biomolecules that exhibit strong cooperative effects on molecular associations are identified as DNRS-signaling biomarkers for further functional analysis. To demonstrate the effectiveness of DNRS in both bulk and single-cell data, the proposed method was applied to a number of datasets, including a simulated dataset, three single-cell datasets of embryonic development and three tumor datasets from The Cancer Genome Atlas (TCGA). Specifically, cell fate commitment was successfully detected in three single-cell datasets of embryonic development, including the differentiation of human embryonic stem cells to definitive endoderm cells (hESC-to-DEC data), the development of epithelial basal cells to the mouse hair follicle (EBC-to-MHF data) and the differentiation of human embryonic stem cells to neurons (hESC-to-neuron data). For three tumor datasets, we identified the pre-transition state before lymph node metastasis (Stage II) for colon adenocarcinoma (COAD), the pre-transition state before the tumor invaded the renal vein (Stage II) for kidney renal clear cell carcinoma (KIRC) and the pre-transition state before distant metastasis (Stage IIIB) for lung adenocarcinoma (LUAD). The identified critical states all agreed with the experimental observations, suggesting the robustness of the DNRS method. In addition, the functional and pathway enrichment analyses revealed that the corresponding DNRS signaling genes were implicated in important biological processes.

2 Materials and methods

2.1 Theoretical background

The theoretical background of the DNRS approach is based on our recently proposed DNB theory. Generally, from a system perspective, the progression of a complex biological system is described by the dynamic evolution of a high-dimensional non-linear system, where a drastic or qualitative shift in a biological process is regarded as a phase transition at a bifurcation point (Shi *et al.*, 2021). In terms of the DNB theory (Chen *et al.*, 2012; Liu *et al.*, 2019), when the system is in the vicinity of a critical point (bifurcation point), a dominant group of biomolecules defined as the DNB appears, which satisfies the following three properties (Chen *et al.*, 2012):

- The variation in each DNB internal biomolecule rapidly increases;
- The correlation between each pair of DNB internal biomolecules drastically increases;
- The correlation between a DNB internal molecule and any external biomolecule drastically decreases.

From the properties of DNB molecules, as the system is close to the critical point, a group of highly correlated and widely fluctuating variables emerges and exhibits strong cooperative effects on molecular associations, signaling the upcoming critical transition. By exploring the dynamic changes of such a group of dominant variables in molecular associations at the network level, it is possible to predict the qualitative state transition of a system. The proposed DNRS method is designed to quantify the dynamic changes in gene cooperative effects of a time-specific directed network, which captures the criticality of biological systems.

To describe and measure the significant changes in gene cooperative effects at a network level, the PageRank method can be employed to quantify the dynamic changes of molecular associations. Let $G = (V, E)$ be a directed graph with K nodes. Denote $A = (a_{ij}) \in \mathbb{R}^{K\times K}$ as the transition matrix of the graph G, and define $d^{out}(i)$ as the out-degree of node i. For node i (corresponding to row i), there are two settings corresponding to two different cases: (i) when $d^{out}(i) > 0$, $a_{ij} = 1/d^{out}(i)$ if node i points to node j in graph

G, and 0 otherwise; (ii) when $d^{out}(i) = 0$, $a_{ij} = 1$ if $j = i$, and 0 otherwise. For such graph G, the PageRank vector $\vec{s}^{*}$ (the PageRank score of nodes) is obtained by solving the fixed point of the following iteration equation (Langville and Meyer, 2011):

$$\vec{s}^{(l+1)} = \alpha A' \vec{s}^{(l)} + \frac{(1-\alpha)}{K}, \tag{1}$$

where α is a damping factor that is usually set as 0.85, and the symbol "′" represents the transpose of a matrix. Taking into account the statistical properties of DNB theory, a personalized PageRank is proposed and used in this study as follows:

$$\vec{s}^{(l+1)} = \alpha H_T' \vec{s}^{(l)} + \alpha\left(\vec{d}' \vec{s}^{(l)}\right)\vec{\tau} + (1-\alpha)\vec{\tau}, \tag{2}$$

where the matrix H_T (as described in Step 2 of the following DNRS algorithm) represents the personalized transition matrix constructed from a time-specific directed network of a time point T. The vector $\vec{d}$ (as presented in Step 3 of the DNRS algorithm) could be used for balancing errors caused by isolated nodes that have no edge pointing to other nodes. Vector $\vec{\tau}$ (as described in Step 4 of the DNRS algorithm) represents a personalized vector that satisfies $\sum_{i=1}^{K} \tau_i = 1$.

The time-specific directed network at a time point T is constructed based on an information-theoretic scheme (Sun *et al.*, 2019), which provides a direction determination index (as defined in Eq. 3) to evaluate the combined effect of gene combinations over a single gene from the perspective of mutual information (MI). Specifically, vectors $\vec{U}$ and $\vec{V}$ are denoted as the expression profile of g_i and g_j in all cells/samples from two adjacent time points T and $T-1$, respectively. Vector $\vec{Y}$ is denoted as a binary phenotype label indicating the sampling time point (T or $T-1$) of each cell, i.e. $_k = T$ means that the kth cell/sample is obtained from the sampling time T, while $_k = T-1$ marks the other case. Let vectors $\hat{X}$ and $\vec{X}$ be defined as $\hat{X} = (\vec{U} + \vec{V})/\sqrt{2}$ and $\vec{X} = \vec{U}$, respectively. Then, whether there is a direction for edge (g_i, g_j) from gene g_i to g_j is decided by the direction determination index $_{i,j}$ defined as follows.

$$\begin{aligned} _{i,j} = &\sum_{\hat{} \in \vec{\hat{X}}} \sum_{\in \vec{Y}} p(\hat{},) log \frac{p(\hat{},)}{p(\hat{})p( )} \\ &- \sum_{\in \vec{X}} \sum_{\in \vec{Y}} p(,) log \frac{p(,)}{p( )p( )}, \end{aligned} \tag{3}$$

where $p(\hat{},)$ is the joint probability density function (pdf) of $\vec{\hat{X}}$ and $\vec{Y}$ and $p(,)$ is the pdf of $\vec{X}$ and $\vec{Y}$. $p(\hat{})$, $p( )$ and $p( )$ represent the marginal pdfs of $\vec{\hat{X}}$, $\vec{X}$ and $\vec{Y}$, respectively. The positive determination value infers that the integration of gene g_j can be considered to be an improvement to the mutual information (MI) of gene g_i; that is, there is a directed edge (g_i, g_j) from gene g_i to g_j in the time-specific directed network.

If the variables follow Gaussian distribution or binomial distribution, Eq. (3) can be expressed as follows (see Supplementary Information C for derivation).

$$_{i,j} = -\frac{1}{2} log \frac{1 - \mathrm{PCC}(\vec{\hat{X}}, \vec{Y})^2}{1 - \mathrm{PCC}(\vec{X}, \vec{Y})^2}, \tag{4}$$

where $\mathrm{PCC}(\vec{\hat{X}}, \vec{Y})$ represents the Pearson correlation coefficient (PCC) between the vector $\hat{X}$ and $\vec{Y}$, and $\mathrm{PCC}(\vec{X}, \vec{Y})$ is the PCC between $\vec{X}$ and $\vec{Y}$. From the properties of the DNB, when the system is close to the vicinity of the critical point, there are much more directed edges appearing for some nodes (i.e. the DNB members) in the time-specific directed network.

2.2 Algorithm to identify the critical point based on DNRS

As mentioned above, for a biological system with K genes/variables, the state of the system at each time point can be revealed by the dynamical changes of cooperative effects on molecular associations. The following algorithm is proposed to explore such dynamical changes at a network level and identify the critical state or tipping point.

[Step 1] Construct the time-specific directed network $N^{(T)}$ at each time point $t = T$ ($T \geq 2$). Based on the protein–protein interaction (PPI) network and cells/samples from two adjacent time points (e.g. N and L cells/samples at the time point T *and* $(T-1)$, respectively), the time-specific directed network $N^{(T)}$ can be constructed by a direction determination index $_{i,j}$ which is defined as Eq. (4). Specifically, if $_{i,j}$ is greater than zero, there is a directed edge (g_i, g_j) from gene g_i to g_j; otherwise, there does not exist a directed edge (g_i, g_j). By this way, we construct a time-specific directed network $N^{(T)}$, where each directed edge (g_i, g_j) from gene g_i to g_j is decided by direction determination index $_{i,j}$.

[Step 2] Construct the network's transition matrix $H_T = (H_{i,j})_{K\times K}$ based on the time-specific directed network $N^{(T)}$, where K represents the number of nodes/genes. The matrix element $H_{i,j}$ represents the direction determination value $_{i,j}$ if there exists a directed edge from the node i (gene g_i) to j (gene g_j), otherwise $H_{i,j}$ is set as 0. Then the standardization of matrix H_T is as follows:

$$H_{i,j} = \frac{_{i,j}}{\sum_{j=1}^{K} _{i,j}} \; if \; \sum_{j=1}^{K} _{i,j} \neq 0 \tag{5}$$

or

$$H_{i,j} = \begin{cases} 0 \; (j \neq i) \\ 1 \; (j = i) \end{cases} if \; \sum_{j=1}^{K} _{i,j} = 0 \tag{6}$$

[Step 3] Build a K-dimensional vector $\vec{d}$, which can be used for balancing errors caused by isolated nodes which have no edge pointing to other nodes. Its elements can be defined as the following criteria.

$$d_i = \begin{cases} 1 & \sum_{j=1}^{K} _{i,j} = 0 \\ 0 & \sum_{j=1}^{K} _{i,j} \neq 0 \end{cases} \quad i = 1, 2, \ldots, K \tag{7}$$

[Step 4] Build a K-dimensional personalized vector $\vec{\tau}$, where the element τ_i represents the standard deviation of node i (gene g_i) based on the gene expressions of N cells at sampling time point T. The normalized vector $\vec{\tau}$ can be obtained by setting each of its element as $\tau_i = \tau_i / \sum_{i=1}^{K} \tau_i$.

[Step 5] Calculate the PageRank vector $\vec{s}^{*}_{T} = (s_1^*, \ldots, s_K^*)$ (presented as Eq. 2) for the time-specific directed network $N^{(T)}$, that is, the local DNRS (gene-specific local DNRS) is calculated for each gene of the time-specific directed network $N^{(T)}$. Then the DNRS for the whole network can be obtained from the following formula:

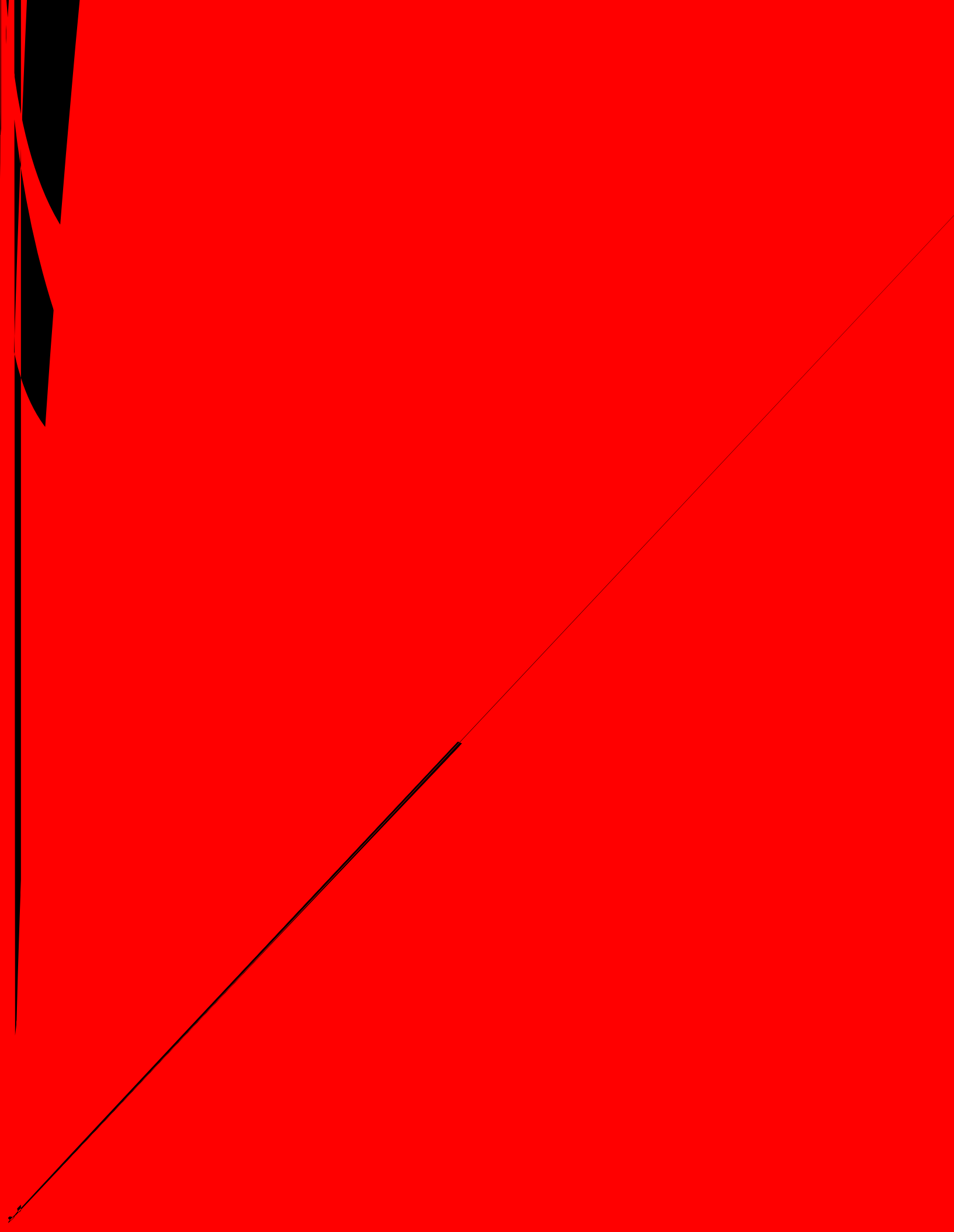
$$PR(T) = \frac{1}{Q} \sum_{i=1}^{Q} \left(\frac{s_i^*}{mean(\vec{s}^{*}_{T})} \right), \tag{8}$$

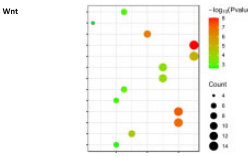
where Q is the number of the top 5% genes with the largest local DNRS and the symbol $mean(\vec{s}^{*}_{T})$ represents the mean of the PageRank vector $\vec{s}^{*}_{T}$.

[Step 6] The critical point is identified by the one-sample t test (Rochon and Kieser, 2011), which is employed to. To analyze how well the DNRS recapitulates the abrupt transition, the one-sample t test index Z is used to determine whether value is significantly different from the mean of n-dimensional vector $X = (_1, _2, \ldots, _n)$, namely,

$$Z = \frac{mean(X) - }{s/\sqrt{n}}, \tag{9}$$

where $mean(X)$ represents the mean of vector X and the s is the standard deviation of vector X. The P-value related to index Z is derived from the t-distribution to assess the statistical difference between $mean(X)$ and . There is a statistically significant difference between $mean(X)$ and if $P < 0.05$. In this study, the time point $t = T$ is viewed as a critical point if $PR(t)$ satisfies two criteria: (i) $PR(t) > PR(t-1)$; (ii) $PR(t)$ is statistically different ($P < 0.05$) from the prior values (also see Supplementary Information D).





that in cancer progression, maintaining cell survival is as a priority for cancer cells before further malignant features such as cell proliferation and tissue infiltration. The presence of stage inconsistencies in the emergence of cancer-associated abnormal cellular functions may be the key to stage-specific precision therapy.

4 Discussion

It is important to hunt for the critical state of complex biological systems, such as the pre-deterioration stage of tumor disease and cell fate commitment during embryonic development. Detecting early warning signals for critical transition just before disease deterioration may provide appropriate timing to prevent or at least prepare for catastrophic deterioration. Understanding the cell fate decision may enable the construction of individual-specific disease models and the design of specific therapies for complex diseases relevant to cell differentiation (Peltier and Schaffer, 2010). However, it is usually challenging to identify the critical transition of complex biological systems since there is little change in the system state before reaching the tipping point. In addition, real biological datasets are sometimes too noisy to characterize the dynamics of biological processes.

In this study, different from traditional methods based on the information of differential expressions, we developed a computational method to explore the dynamic changes in cooperative effects on molecular associations, thus signaling an upcoming critical transition when the complex biological system is close to the tipping point. The proposed DNRS has been successfully applied to both a numerical dataset (Fig. 2) and six real biological datasets (Fig. 3). Specifically, the abrupt increase in the DNRS demonstrates the tipping point (E13.5) of the EBC-to-MHF process before differentiation into the mouse hair follicle, the tipping point (36 h) of the hESC-to-DEC process before differentiation into the definitive endoderm, the tipping point (Day 26) of the hESC-to-neuron process before differentiation into neuronal cells, the critical state (Stage II) of COAD before lymph node metastasis, the critical state (Stage II) of KIRC before the tumor invades the renal vein, and the critical state (Stage IIIB) of LUAD before distant metastasis. In addition, functional enrichment analysis revealed that the common DNRS signaling genes for three cancer datasets and two human embryonic differentiation datasets are involved in significant biological processes or pathways (Figs 5 and 6). However, DNRS may not perform well if there are too few samples at a specific time point. In addition, the time-specific directed network is constructed based on a priori knowledge-based PPI network.

To summarize, there are the following advantages of the proposed method. First, compared with the classical DNB, the DNRS is more sensitive to critical signals (see Supplementary Information H for details). Second, compared to the traditional biomarkers that are used for the detection of the after-transition state based on the information of differential expressions, the DNRS method can detect early-warning signals for the pre-transition state just before the catastrophic transition by exploring the dynamic changes of in cooperative effects on molecular associations. Third, it is noteworthy that the DNRS is model-free and different from conventional machine learning models; that is, it requires neither feature selection nor parameter training. Together with the dynamic prediction method (Chen *et al.*, 2020), the DNRS may not only detect the criticality of a system, but reveal the dynamic change in molecular associations that may drive the system into an irreversible state transition.

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Conflict of Interest: none declared.

Data availability

EBC-to-MHF data (ID: GSE147372), hESC-to-DEC data (ID: GSE75748), and hESC-to-neuron data (ID: GSE86977) are accessible from the NCBI Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). Colon adenocarcinoma (COAD), kidney renal clear cell carcinoma (KIRC), and lung adenocarcinoma (LUAD) are accessible from the cancer genome atlas (TCGA) database (http://cancergenome.nih.gov).

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