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Review

Cancer reversion, a renewed challenge in systems biology

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Abstract

Cancer is a complex disease for which conventional therapeutic approaches often encounter a fundamental limitation. As an alternative approach, there is a renewed challenge in systems biology for cancer reversion by converting cancer cells into normal cells. Historically, such reversion has been observed sporadically, but no systems analysis has been attempted so far. We review the phenomenal observations of cancer reversion in history and introduce two relevant systems biological approaches based on molecular network modeling. We further introduce the recent development of network control strategies that can be used to identify useful molecular targets for cancer reversion and then discuss future challenges in systems biology.

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Keywords

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Introduction

Cancer is becoming more important as our society is getting aged [1]. There is, however, a fundamental limitation in cancer treatment despite the recent development of targeted therapy and immunotherapy [2,3]. The goal of conventional cancer therapy is to induce apoptosis of cancer cells. The ultimate limitation of this approach lies in that cancer cells are still a part of ourselves and therefore we cannot selectively remove them without damaging normal cells. Can we consider then an alternative approach other than inducing apoptosis? We propose reversing cancer cells into normal cells instead of directly killing them. Such concept of cancer reversion is not new [4], but there is a renewed challenge in the era of systems biology.

Historically, the phenomena of cancer reversion have been observed sporadically [5], but the underlying mechanism has not been understood and no systems analysis was attempted. From a systems biological perspective, cancer can be viewed as a network disease caused by dysregulation of the dynamics of an intracellular molecular regulatory network [6]. Thus, considering the huge dimensionality and functional redundancy of the molecular network, we might be able to restore the network functionality of normal cells by controlling some of the molecular targets in the network.

In this review, we first review the historical observations of cancer reversion (Table 1). Then, we introduce two systems biological approaches for cancer reversion: data-driven statistical network modeling approach and mechanism-based logical modeling approach. We further review the recent development of network control in order to identify useful molecular targets for cancer reversion based on network models. Finally, we discuss the future challenge of systems biology for cancer reversion.

History of cancer reversion

The first observation of cancer reversion was reported in 1907 [7]. It was about the phenomenon that ovarian teratoma was spontaneously differentiated into a normal somatic cell lineage. Since then a number of similar phenomena have been occasionally reported, not only in mammals, but also in plants, newts, and other various organisms [8,9,31]. Among them, the most important evidence for cancer reversion was the discovery by Mintz et al. in 1975 that blastocysts injected with embryonal carcinoma cells were successfully developed into normal organs and tissues [12]. This clearly implicates that cancer cells can be reverted to normal cells that have controlled proliferation and regular tissue-specific functions. Not only the embryonal carcinoma, a specific cancer cell type not necessarily harboring somatic mutations, but also other cancer cells with somatic mutations or aneuploidy were observed to be revertible to normal states [32,33].

Table 1

Summary of the history on cancer reversion.

Year	Descriptions	Reference
Early discoveries		
1907	Ovarian teratoma cells were differentiated into normal-like cells. **The first observation related to cancer reversion.**	[7]
1951	Plant tumors could recover their normal phenotypes through sequential transplantation into healthy plants.	[8]
1965	Hamster cells transformed by Rous Sarcoma Virus were partially converted to non-tumorigenic cells showing the growing pattern of untransformed cells. **This observation implied that the cancer with irreversible alterations such as mutation and oncogene amplification might be reversible to normal-like states.**	[9]
1968	Survived cancer cells after FUdR treatment could achieve morphologically normal phenotypes (these cells were called 'flat revertant') and lost their colony forming capability *in vitro*.	[10]
1973	Embryonic mammary mesenchyme induced the differentiation of mouse breast cancer cells.	[11]
1975	Normal genetically mosaic mice were successfully developed from blastocysts injected with malignant teratocarcinoma. **This study suggested that the teratoma injected in blastocysts might develop to any type of tissues and could produce functional germ cells.**	[12]
Microenvironmental changes		
1997	Three dimensional culture with integrin-blocking antibody successfully reversed human breast cancer cells into non-malignant cells.	[13]
1998	Mouse liver cancer cells were differentiated into normal hepatocyte in splenic microenvironments.	[14]
2008	Nodal-inhibition triggered the reversion of human melanoma cells toward normal melanocytic phenotypes. **This study showed that embryonic microenvironments might effectively suppress malignancy and differentiate cancer cells such that they have normal phenotypes.**	[15]
Direct differentiation		
1988	**The first clinical trial of ATRA in patients with APL. All 24 participants of the trial showed a complete remission.**	[16]
1998	Inhibition of PPAR-γ caused the differentiation of human colorectal cancer *in vitro* and *in vivo*.	[17]
2001	HDAC inhibitors effectively blocked the proliferation of various human breast cancer cells and successfully differentiated them into morphologically normal cells.	[18]
Oncogene addiction		
1999	The tumorigenesis induced by Myc-hyperactivation in hematopoietic lineages was reversed to their original non-tumorigenic states by inactivation of Myc.	[19]
2000	**The term 'oncogene addiction' was first proposed to explain the death or differentiation of cancer cells by inhibition of a single oncoprotein.**	[20]
2000	Ablation of Bcr-Abl in acute B-cell leukemia reversed cancers cells without apoptosis and showed complete remission in a mouse model	[21]
2007	Suppressed Myc expression rescued intestinal neoplasia caused by Apc loss.	[22]
2015	Apc restoration re-established a normal crypt-villus structure in intestinal carcinoma. **This study showed that the reversed cells can recover the normal function of intestinal cells and make a balance between self-renewal and differentiation.**	[23]
Direct reprogramming		
2004	Human melanoma cells were reprogrammed into normal pluripotent stem cells by nuclear transplantation. The reprogrammed cells were then normally differentiated into multiple cell types such as melanocytes, lymphocytes, and fibroblasts. **The first nuclear reprogramming study using cancer cells.**	[24]
2010	Gastrointestinal cancer cells were reprogrammed into induced pluripotent stem cells that have slowly proliferating characteristics and reduced tumorigenicity.	[25]
2013	Induced pluripotent stem cells derived from glioblastoma were re-differentiated into malignant neuronal progenitor cells, but they became nonmalignant cells when differentiated into non-neuronal lineages.	[26]
2015	Acute lymphoblastic leukemia cells were transformed to non-malignant macrophages when exposed to myeloid differentiation-promoting cytokines	[27]
Other methods		
1989	Krev-1 reduced malignancy by converting cancer to flat revertants that have relatively normal-like phenotypes such as reduced proliferation and lowered tumor-producing capability *in vivo*.	[28]
1993	The revertant cells derived by H-1 parvovirus, the specialized type of virus preferentially killing cancer cells, showed significantly lower tumorigenicity *in vitro* and *in vivo*.	[29]
2002	Comparison of the gene expression profiles between flat revertant cells and their original cancer state cells revealed that SIAH1 and tpt1/TCTP might be the revertant-inducing factors.	[30]

FUdR, floxuridine; ATRA, all-trans retinoic acid; APL, acute promyelocytic leukemia; PPAR-γ, peroxisome proliferator-activated receptor gamma; HDAC, histone deacetylase.
Significance and implication of the studies are highlighted in bold.

Subsequent to these early discoveries, three major research streams associated with cancer reversion were independently developed since 1980s. First, microenvironmental conditions for cancer reversion were investigated. Interestingly, embryonic microenvironments are found to be important to reverse many cancer cell types such as breast cancer, prostate cancer, and melanoma [34]. For instance, Nodal inhibition was considered as the direct molecular mechanism that causes melanoma reversion by observing the difference between embryonic microenvironment and cancer microenvironment. The major difference was the existence of Nodal antagonizing factors in embryonic microenvironment that inhibit Smad2/3 signaling pathways [15,35]. In addition, Weaver et al. found that integrin blocking can successfully reverse breast cancer cells to normal-like cells using 3D culture [13]. These indicate that alteration of microenvironments could reverse tumorigenecity by modulating extrinsic factors such as extracellular matrix and TGF-β superfamily. Another approach was differentiation therapy. For instance, retinoic acid (RA) was found to differentiate cancer cells into non-proliferative cells [36]. Its efficacy was profound for acute promyelocytic leukemia (APL), and the subsequent transcriptomic and proteomic data analysis suggested its potential mechanism as activation of calcium, interferon, and proteasomal signaling pathways [37]. Notably, its clinical trials on APL showed complete remission of cancer even for those who had resistance to previous chemotherapy [36]. In addition, peroxisome proliferator-activated receptor gamma (PPAR-g) and histone deacetylases (HDACs) were also found to be such differentiating factors in colorectal cancer and breast cancer, respectively [17,18]. The third approach was based on the concept of oncogene addiction. In this approach, *Myc* inactivation was found to induce growth arrest or differentiation in various types of cancer such as lymphoma, osteogenic sarcoma, skin papilloma, and islet-cell adenocarcinoma [38]. Recently, it was found that *Myc* deletion can revert cancerous intestinal tissues to healthy normal crypt-villus structures in mice [22].

While the three main approaches were continuously extended, another promising approach was suggested from the stem cell research field since 2000s. It was the reprogramming technology that unprecedentedly facilitated fate conversion from a certain cell type to another. Intriguingly, induced pluripotent stem cells derived from cancer cells seemed to be normal even when they were further differentiated into particular cell lineages [24,25,39]. For instance, Zhang et al. observed that reprogrammed sarcoma can be terminally differentiated into bone or fat without tumorigenicity [40]. Such observation implies that the genetic abnormality of cancer cells might be overcome by epigenetic reprogramming. Recent observations show that B cell acute lymphoblastic leukemia could be successfully reprogrammed into non-malignant macrophages [27] and that restoration of *Apc* can revert a cancerous crypt into a normal functional crypt [23].

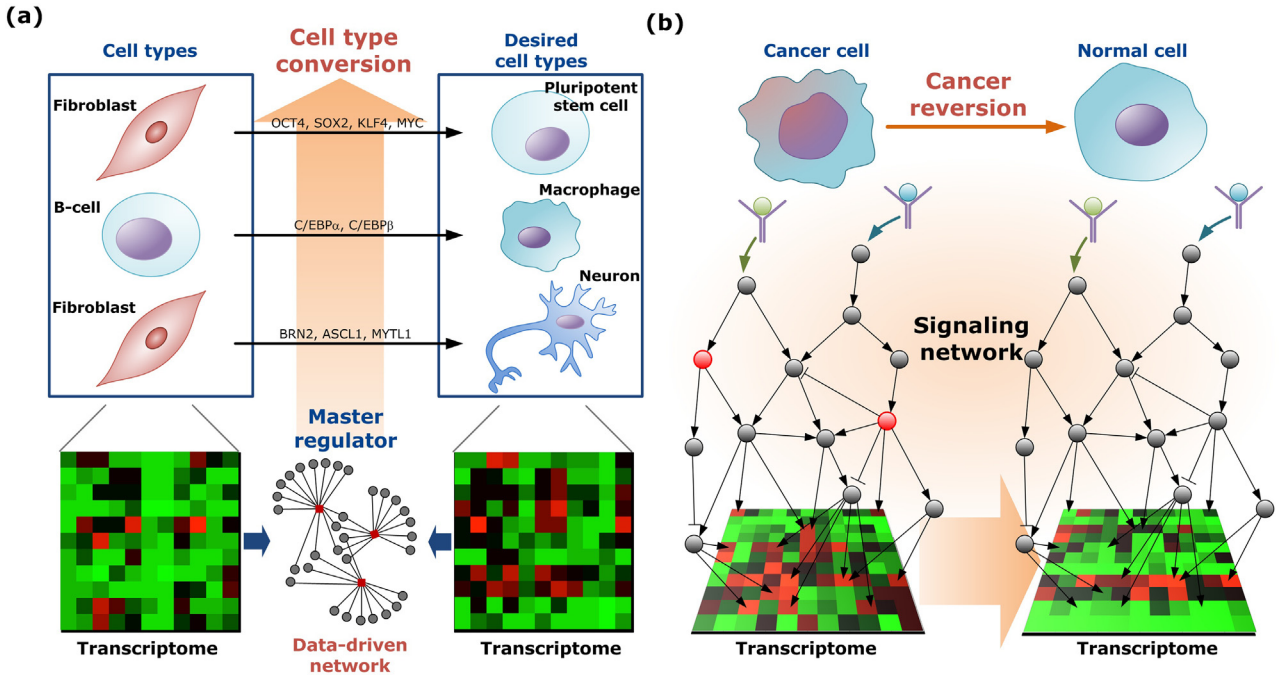
Although aforementioned reversion factors are various molecular components (e.g. cytokines; Nodal, transcription factors; Myc, epigenetic regulators; HDACs, and metabolites; RA), their biological functions are well-known to perform a central role in cell fate decision such as differentiation, development, proliferation, and apoptosis [38,41,42]. This agrees with that a hub node, a central molecule in biological networks, is crucial in biological systems [43]. However, the functional role of reversion factors might depend on cellular context and thereby the precise molecular mechanism still remains mostly elusive. Therefore, systems biological studies on cancer reversion are required not only to identify more promising molecular targets in a systematic way but also to reveal the underlying mechanism at a system-level.

Data-driven statistical network modeling

Although there have been a number of experimental reports showing the possibility of cancer reversion, we should note that most of them focused on a few confined phenotypes such as growth rate, mobility, and survival potential. This means that none of the previous studies actually showed the explicit reprogramming of cancer cells at a molecular level. On the other hand, some recent studies of trans-differentiating cell identity showed the possibility of determining the molecular mechanism of cancer reversion in terms of cellular reprogramming. In particular, some of them employed a data-driven statistical network modeling approach to identify reprogramming factors. For instance, Carro et al. inferred glioblastoma multiforme (GBM) network and converted a mesenchymal subtype into a proneural subtype [44]. Suva et al. also showed that differentiated GBM cells can be reprogrammed to stem-like tumor propagating cells by introducing several neurodevelopmental transcription factors [45].

The recent data-driven approach was motivated by the developmental fate conversion studies [46–49] which share a common basis that cellular reprogramming can be achieved at a transcriptional level (Figure 1a). In other words, cellular identity is determined by the gene regulatory network and the master regulators that are at the top of the regulatory network [50], and each molecular state corresponding to a certain phenotype can be inferred from gene expression profiles. These studies are exemplary frameworks of inferring gene regulatory networks, identifying master regulators for specific cellular identities, and converting cell identities upon these frameworks [47–49]. Similar approaches were also applied to cancer cells to identify causal driver genes and to displace the cancerous identity [44,51,52]. Among them, Carro et al. identified two transcription factors (C/EBPβ and STAT3) as master regulators based

Figure 1



Data-driven statistical network modeling approach. (a) Previous studies on cell type conversion based on the data-driven statistical network modeling approach. The origin of cell type conversion study can be traced back to the reprogramming of adult fibroblasts to induced pluripotent stem cells by extrinsic overexpression of Oct4, Sox2, Klf4 and Myc [46]. Since then a number of case studies on hematopoietic, neuronal, and myocardial lineages and other numerous developmental cell types were conducted by overexpressing several master regulators identified by the data-driven approach [47–49]. In the figure, a cell type is represented by a unique expression profile and it was presumed that a few master regulators govern the whole transcriptomic landscape. To find out these master regulators, we can employ data-driven statistical network inferences that were developed primarily focusing on the correlation of expressions. Since steady-state gene expression profiles were mostly considered in this case, we can only infer directed acyclic networks. **(b)** Illustration of cancer reversion at a network-level. Like most other developmental cell fates, both cancer and normal cellular states can be represented by their unique gene expression profiles. However, the aberration in signaling pathway molecules, which is the critical factor distinguishing between cancer and normal cellular states, should be investigated at multi-dimensional aspects.

on the fact that their gene expression patterns are highly associated with mesenchymal genes of GBM and that they are at the top of the hierarchical transcriptional regulatory network [44].

This data-driven approach, or reverse engineering, presumes that cellular phenotypes display their own molecular profiles at steady states, and a few master regulators of each steady state can control the whole transcriptomic landscape. Hence, the data used for network inference in this approach are mostly steady-state gene expression profiles and therefore the inferred network represents statistical associations between molecules.

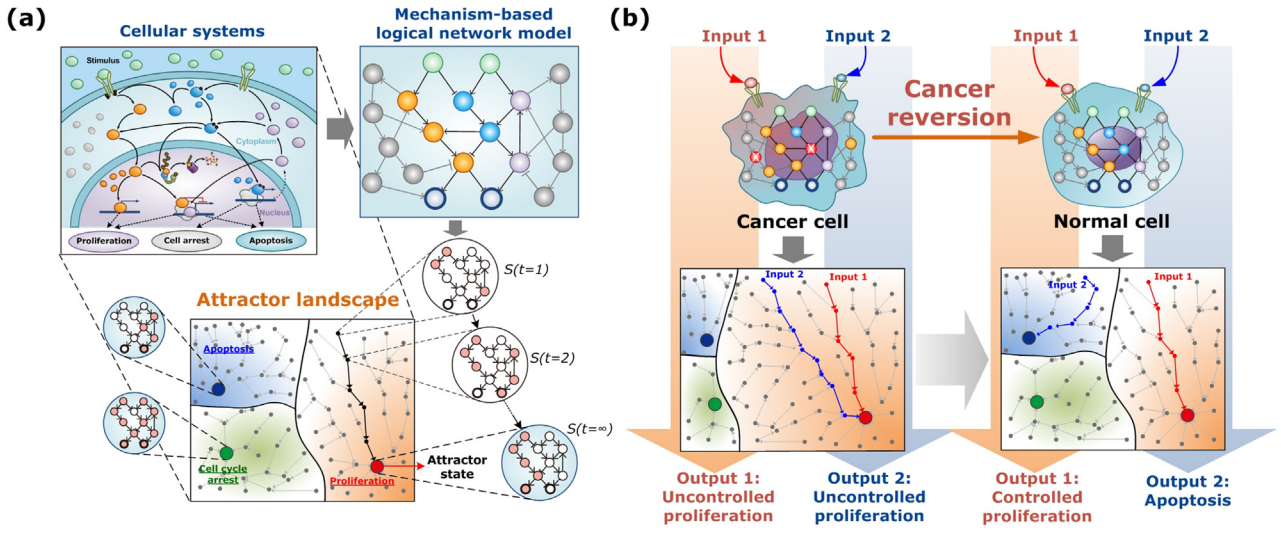
An important advantage of data-driven statistical network modeling approach is that the resulting network can be of genome-wide scale without any bias and represent a cell-type specific context. As more data are being accumulated in life sciences, this data-driven approach would become a more powerful tool to establish the reprogramming technology. However, the data-driven statistical network modeling approach has a fundamental limitation in identifying direct causality and taking account of the feedback regulation among biomolecules. This critically affects inferring signaling pathways which contain many complex regulations including feedback loops. Considering that most molecular aberrations in cancer occur at a proteomic level, particularly for signaling molecules, we can infer that normal and cancerous states have demarcation at a multi-dimensional level including not only transcription factors, but also signaling proteins and epigenetic regulators (Figure 1b). In this regard, we note that some recent studies figured out hidden regulatory molecules beyond the transcription factors using integrative frameworks [51]. Moreover, recent studies on network modeling based on phosphoproteome or metabolome enable us to identify such master regulators that can determine not only gene expression levels but also metabolic and proteomic states [53,54]. Therefore, multi-dimensional omics data-driven modeling will be crucial for cancer reversion. In summary, the data-driven statistical network modeling approach showed remarkable achievements in converting developmental cell fates, but still has a lot of challenge to be used for cancer reversion for which we need to consider more sophisticated regulatory mechanisms.

Mechanism-based logical network modeling

A data-driven statistical network model can provide us with a snapshot of the particular cell phenotype, but not the dynamical function of a cellular system in consideration of input–output relationships. Therefore, there is a fundamental limitation in dealing with the functional difference between normal and cancer cells with respect to the dynamical aspect using the data-driven statistical network model. A biological function can be represented by an input–output mapping of the cellular system. For instance, typical hallmarks of cancer such as insensitivity to anti-growth signals and evading apoptosis are the examples of different outputs of cancer cells from normal cells to the same input signals [55]. To address such dynamic properties of a cellular system, a mechanism-based logical network model is needed (Figure 2a). It can be constructed by integrating all the experimental findings about biochemical interactions between molecules where each link in this model represents a real causal relationship. Using this model, we can investigate the dynamic change of each molecular activity that is determined by the complex regulation of the network. When we consider the overall network state change and investigate its converging dynamics, an attractor landscape analysis is often useful where an attractor represents a final steady state or a set of cyclic states to which a given initial state converges. Attractor states of a molecular regulatory network are determined by the wiring pattern and regulatory logics among the molecules. It is well known that negative feedback can induce an oscillatory behavior through a cyclic attractor whereas positive feedback can induce multi-stationarity by resulting in multiple stable points [56,57]. Hence, attractor states of a network can be changed by perturbing potential regulatory molecules or regulatory logics of the feedback loop. For instance, negative feedback loops of p53 through Mdm2 and Wip1 contribute to the oscillatory behavior of p53 in response to DNA damage by activating a cyclic attractor that corresponds to cell cycle arrest. In this case, by disrupting the negative feedbacks with Mdm2 or Wip1 inhibition, the sustained activation of p53 can be induced through a point attractor state that represents apoptosis [58]. The attractor landscape consists of all the attractors as well as their basin of attraction. By including the inputs to a cellular system as a part of the network nodes, the input–output relationship can also be represented in the attractor landscape.

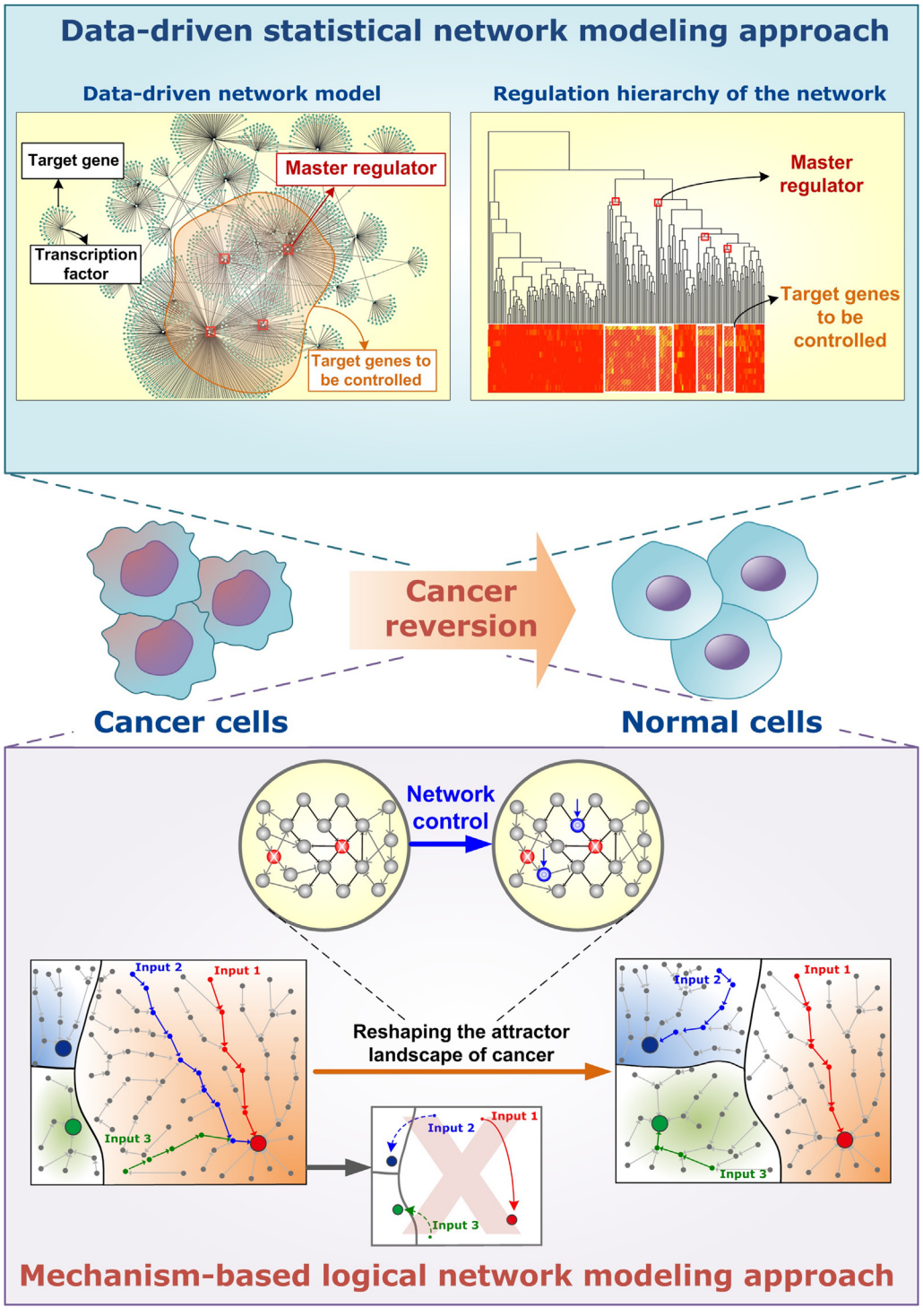
The logical network model can be employed to investigate the hidden mechanism underlying the cancer reversion. Some relevant studies were reported recently. For instance, Fumia et al. reconstructed a Boolean network model of cancer cells and showed how cancer cells can produce different responses than normal cells to the same input according to their internal states [59]. In addition, Choi et al. showed how normal breast cells

Figure 2



Attractor landscape analysis for cancer reversion using mechanism-based logical network modeling. (a) A cellular system and its attractor landscape of the underlying molecular interaction network. The cell consists of numerous molecules that are interacting with each other to form a huge dynamic interaction network. The interaction between molecules constrains each molecular activity and the network dynamics driven by such interactions determine the network state (i.e. a collection of the activity levels of molecules) which eventually converges to a (pseudo-) steady state, or attractor. The attractor is determined by inherent dynamics of the network as well as the initial state which can also include the input values. An attractor landscape of a cellular system consists of all attractors and their basin of attraction. Pr, Ar, and Ap stand for proliferation, arrest, and apoptosis, respectively. **(b)** Illustration of differential landscapes of normal and cancer cells. Normal and cancer cells exhibit different cellular identities, such as input–output relationships, since they have different attractor landscapes even though they have the same attractors. In this respect, cancer reversion can be interpreted as a recovery process toward the attractor landscape of a normal cell.

Figure 3



Identifying control targets for cancer reversion based on the study of complex network control. (top) For data-driven statistical network models, the network control problem is to identify master regulator(s) that can cover maximal target genes to be controlled while maintaining minimal influences on off-target genes. The data-driven statistical network models are usually in the form of a directed-acyclic graph having a hierarchical structure. Hence, the master regulators in the top hierarchy may regulate many off-target genes whereas the regulators in the low hierarchy may not sufficiently cover the target genes to be controlled. The key issue is therefore to identify optimal master regulator(s) that can make a balance between such specificity and sensitivity. **(bottom)** For mechanism-based logical network models, previous studies on the network control have usually focused on the transition between attractors in a given attractor landscape. However, for cancer reversion, we need to develop a new control strategy by which the attractor landscape of cancer can be reshaped to restore the input–output relationship of the normal cell.

and breast cancer cells differently respond to the same DNA damage signal by analyzing their attractor landscapes [58]. The dynamical input–output cellular responses of urinary bladder cancer and colorectal cancer were also investigated using the logical network model [60,61]. These examples demonstrate the potential applicability of the mechanism-based logical network model to the systems biological study of cancer reversion with a particular focus on signaling pathways [62]. Recently, some niche factor requirements were revealed to be critical in distinguishing between colon epithelial cells and colon cancer cells, which indicates that niche factors such as Wnt and epidermal growth factors (EGFs) are crucial for normal epithelial maintenance but not in cancer cells [63]. Together, the attractor landscape analysis of a mechanism-based logical network model might be useful for revealing the hidden mechanism of cancer reversion and establishing a systematic strategy for it [64] (Figure 2b).

Despite the aforementioned potential applicability, the mechanism-based logical network modeling has also limitations. Although many molecular interactions were revealed over last two decades, there are still some unknown interactions to be further discovered which will constitute an uncertainty of the resulting model. Another difficulty is reflecting a detailed cellular context to the model where the contextual information should be obtained from *in situ* analysis. We can overcome these limitations by combining the mechanism-based logical network modeling with the data-driven statistical network modeling [65,66].

Network control strategy

We reviewed two different approaches for network modeling that can be used for cancer reversion. Choosing an appropriate modeling depends on how to define the normal and cancerous cellular states. In any case, we ultimately arrive at a network control problem, identifying control target(s) in the network for cancer reversion.

The control problem upon the data-driven statistical network model is to find out a master regulator where the perturbation of which subsequently regulates all of its target genes. In this case, the master regulator is generally a hub node located at a top in the hierarchy of the subnetwork (Figure 3, top). A few algorithms were developed to infer such master regulator that determines a specific cellular identity [48,49]. The major issue in this case is optimizing the balance between sensitivity and specificity of the network control. For instance, controlling the master regulator of the highest network hierarchy can achieve high sensitivity but would result in low specificity. To resolve this problem, we can make use of the recent developments in the field of complex network control [67]. Liu et al. applied the structural controllability to directed complex networks and developed an efficient method which can be used to identify a minimal set of driver nodes for controlling any network state to a desired state [68]. We can further apply this idea to identify useful control targets for cancer reversion.

On the other hand, the mechanism-based logical network model describes the nonlinear dynamics of a cellular system. In this case, the attractor landscape analysis might be useful to investigate the overall difference between normal and cancerous cellular states in order to further develop a control strategy for cancer reversion. Recently, some remarkable studies were conducted in this framework which suggested various control strategies by iteratively perturbing network nodes [69] or links [70], or by pinning some molecular activities of nodes [71]. For instance, Cornelius et al. suggested a control strategy that can drive a cancerous or precancerous network state to an apoptosis state upon the T-cell survival signaling network model [69]. However, for cancer reversion, we might need to reshape the attractor landscape itself instead of simply relocating the network state upon a fixed attractor landscape of cancer cells to recover the functional input–output relationship of normal cells [72]. Here, the attractor landscape of cancer cells can be characterized by a dysregulated cellular response for uncontrolled proliferation regardless of input signals (Figure 3, bottom). For cancer reversion, we might need to rewire the network by constitutively controlling some target nodes or links such that the dynamics of the rewired network are changed, leading to reshaping of the attractor landscape. This remains as a future challenge in systems biology for cancer reversion.

Conclusions

Although the first observation of cancer reversion was reported more than a hundred years ago and many biological evidences have been accumulated so far, the underlying mechanism is still largely unknown and no systems analysis has yet been attempted. We introduced two relevant systems biological approaches for cancer reversion: data-driven statistical network modeling and mechanism-based logical network modeling. Both have advantages and disadvantages. Therefore, combining these two approaches would be an important future challenge in systems biology. Furthermore, there is a pressing need to investigate microenvironmental conditions for cancer reversion. Such microenvironmental conditions can be incorporated as input signals to the network model. Developing multi-scale models by integrating intracellular signaling pathways and extracellular microenvironments remains as a future challenge [73,74]. The network control strategy is also a crucial issue and its development will further accelerate the study of cancer reversion.

Intra-tumor heterogeneity and incomplete network models might be barriers in applying cancer reversion strategy to clinics. To overcome these problems, we could adopt the idea of robust control from control engineering, which is a kind of control method ensuring controllability when a system has uncertain components of structural changes [75]. Moreover, mutational heterogeneity among patients might be another barrier since such heterogeneity could result in different outcome between patients against the same control strategy. To solve this problem, we could develop network modeling approaches combined with patient-derived genomic and molecular information, thereby providing patient-specific strategy for cancer reversion [64]. Altogether, this intriguing and critical subject from a basic science perspective can also provide an alternative paradigm of current cancer treatment from a clinical point of view.

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