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Review

How to escape the cancer attractor: Rationale and limitations of multi-target drugs

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ABSTRACT

The increasingly evident limitations of target-selective cancer therapy has stimulated a flurry of ideas for overcoming the development of resistance and recurrence – the near universal reason for therapy failure from which target-selective drugs are not exempt. A widely proposed approach to conquer therapy resistance is to depart from the myopic focus on individual causal pathways and instead target multiple nodes in the cancer cell's gene regulatory network. However, most ideas rely on a simplistic conceptualization of networks: utilizing solely their topology and treating it as a display of causal interactions, while ignoring the integrated dynamics in state space. Here, we review the more encompassing formal framework of global network dynamics in which cancer cells, like normal cell types, are high-dimensional attractor states. Then therapy is represented by the network perturbation that will promote the exit from such cancer attractors and reentering a normal attractor. We show in this qualitative and accessible discussion how the idea of a quasi-potential landscape and the theory of least-action-path offer a new formal understanding for computing the set of network nodes (molecular targets) that need to be targeted in concert in order to exit the cancer attractor. But targeting cancer cells based on the network configuration of an "average" cancer cell, however precise, may not suffice to eradicate all tumor cells because of the dynamic non-genetic heterogeneity of cancer cell populations that makes them moving targets and drives the replenishment of the cancer attractor with surviving, non-responsive cells from neighboring abnormal attractors.

1. Introduction

The near universal failure of target-selective anti-cancer drugs [1] designed to block a mitogenic or survival pathway has revived an old idea: to use either combination therapy or multi-target drugs (polypharmacology) [2,3] to conquer the cancer cells' unfathomable capacity of resilience and resistance. But what is new? Combination therapy has of course been in use since the dawn of chemotherapy – motivated by clinical reasoning [4]. But the increasing availability of pathway information, which has brought us the target-selective drugs, and burgeoning "systems biology" thinking in drug discovery [5,6] have led to a new climate of multi-dimensional mechanistic reasoning. One central idea is that by attacking multiple pathways at once one could prevent the utilization of alternative survival pathways that allows cells to evade the therapy (for recent review, see [7,8]).

However, there is a central problem with such intuitive but linear-additive thinking which can be traced back to the initial success of molecular biology but has persisted into the modern days of systems biology [9]. The culture of viewing signaling pathways as causal links that connect molecules to the malignant phenotype prevails. It has resisted the insight that these signaling pathways are not independent chains of causation but instead, form a giant, genome-wide molecular network [10]: a gene regulatory network (GRN) [11] in which transcriptional activators modulate the transcription of genes is linked to protein–protein interaction networks [12] between regulatory and effector proteins. Thus, drug action must be viewed as a manipulation of the function of a genome-scale network in its entirety [13].

Based on existing data of connectivity it is reasonable to assume, in a first approximation, that regulatory genes and proteins form a genome wide "*connected component*" as opposed to being organized in disjoint fragments of independent subnetworks [10]. In fact, the perturbation of a regulatory gene product (e.g., via genetic knock-out) leads to "avalanches" of gene expression change consisting of typically 100–1000 s of genes [14]. (An exception would be the blockade of a peripheral effector protein that exerts a

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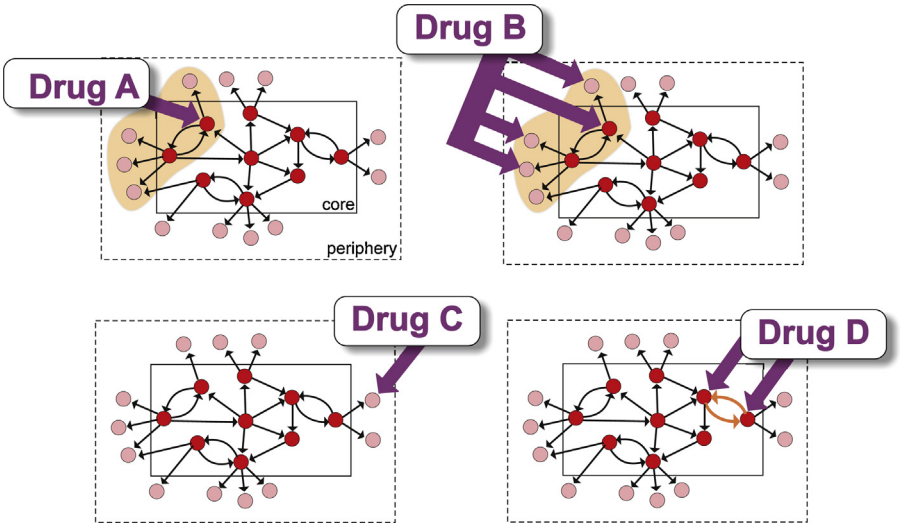

Fig. 1. Principles of effect on molecular network of multi-target and "dirty" drugs and combination therapy: schematic representation of an ideal target-selective drug (*Drug A*) and of a "dirty" (*Drug B*) that targets multiple nodes of the network and has been selected for inducing the same phenotype (e.g., apoptosis) as the one intended for the target-selective *Drug A*. Orange area = spread of perturbation (nearest nodes only) which is almost identical for these two classes of drugs. *Drug C* targets a peripheral node that represents an effector (at the "periphery" of the network, presented by pink nodes), not a regulator protein (in the "core", red nodes). Targeting peripheral nodesl permits a linear mapping between molecular perturbation and phenotype. *Drug D* is designed to strategically target two nodes to achieve attractor exit (see Fig. 3B).

cellular function, such as a metabolic enzyme, and does not regulate other genes or proteins – *Drug D* in Fig. 1).

The genome-scale propagation of the effect of perturbing a specific molecule in the cells' regulatory network presents a *paradox* in view of the idea behind target-selective drugs: if the perturbation of a single node (gene or protein) of a network spreads widely to other nodes of the network, then both a target-selective and a non-selective (multi-target or "dirty" [15,16]) drug will trigger the equivalent of a multi-point perturbation anyway (Fig. 1)! There would be no qualitative difference between a target-selective and non-selective, i.e., "promiscuous" or "dirty" drug (Fig. 1, Drugs A vs. B). Moreover, one may then equally ask: what exactly is gained by combining multiple drugs or by designing multi-target drugs if the effect of a truly single-target drug propagates anyway through the network? Is there a fundamental difference between drugs that directly vs. indirectly hit a large set of targets? Can one optimize the selection of the target set? (We here are concerned about maximizing therapeutic efficacy and leave out the apparently trivial question of minimizing unwanted side effects due to "off-target" activity).

It is well appreciated that many clinically effective drugs are often "dirty drugs" [15,16]: aspirin has at least 20 direct molecular targets and countless more indirect targets downstream of the autacoids it affects [17–19] (see [2,13] for more drug examples). Inhibitors of histone de-acetylation [16] or DNA methylation [20,21], events which control of gene expression across the genome through chromatin modeling, can trigger specific and distinct cell fate switches, such as differentiation, despite their genome-wide action. Thus, in an ironic twist of common notion, the effect of drugs that target specific molecules will spread though the network whereas the effect of drugs that impart non-specific (network wide) perturbations can be channeled to distinct cell-level phenotype changes. Here we will present a conceptual framework that can help to reconcile this paradox.

In another regard we need to distinguish two classes of effects on the network caused by the promiscuous drugs: first, some effects, including negative side-effects, result directly from the perturbation of nodes in the *periphery* of the network (Fig. 1, pink nodes) [22]. Here a *direct linear mapping* between molecule and phenotype is permissive and the phenotype effect of the perturbations of each node can be added-up. Thus, blockade of peripheral *effector* proteins, which are regulated but are not regulating, readily admit prediction of effects. By contrast, a second type of effects emerge from the *joint* action of the perturbed set of connected targets through distributed, non-linear processing of network-wide perturbation [13]. Such *emergent* effects of network targeting will be at discussion here. They can be analyzed only by embracing the theory of global network *dynamics* – a conceptual tool that is rarely used by pharmacologists.

Thus, the goal of this article is to first present in a condensed fashion the concepts of global dynamics of gene regulatory networks and "cancer attractors" [23,24]. They will help explain a series of phenomena associated with the non-genetic (mutation-independent) plasticity of cancer cells that have recently attracted renewed attention (for review see: [25–27]). With this background we will then explain in a qualitative but formally accurate manner the principles for why stimulating a cell to exit the "cancer attractor" state requires a multi-node perturbation and how (in theory) to rationally determine the nodes to be modulated to achieve this. We conclude with discussing the inherent limitations of even the ideal multi-target approach.

2. Failure of current therapy and hope in network approaches

The rapid embrace of network ideas in cancer therapy was stimulated by the near universal failure of cancer therapy, almost uniformly due to development of "acquired" drug resistance and recurrence. The mechanisms can roughly be classified in the four groups: (i) *activation of alternative signaling pathways* [1,28–32]. (ii) *Somatic Darwinian evolution*: e.g., selection of cells with point mutations in the targeted protein that prevent drug-binding [31,33–35]. In a new twist to this classical paradigm, natural selection may be dramatically accelerated by stress-induced mutagenesis (triggered by the drug's cytotoxicity) and genetic assimilation propelled by non-genetic plasticity (see next point) [36,37]. (iii) *Non-genetic heterogeneity and plasticity*: even clonal tumor cells are a heterogeneous population of many phenotypes that convert into each other and vary in their drug responsiveness (see Ref. [37]) – thus allowing for mutation-less selection of cells in more resistant, stem-cell

like states ("persistors"). Even worse, cytotoxic drugs also *induce* the transition of individual cells into such stem-like substrates [28,38–41]. (iv) *Response of the non-tumor* (*stromal*) *cells*: the non-neoplastic tumor stroma may also respond to anti-cancer drugs by remodeling [42] or secretion soluble mediators that confer tumors drug resistance [43–45].

Despite this variety of reasons for drug failure and the holistic ideas of tumor as a disease of the tissue and associated attempts to target the non-cancerous tumor stroma, as most prosaically represented by anti-angiogenesis therapies, adherence to the old paradigm of perturbing the cancer cell itself still endures. It rests on the hope that more efficiently steering the cancer cell's fate toward apoptosis, senescence or differentiation may be possible by considering the entire regulatory network. The arrival of systems biology comes at the right time and has created a new enthusiasm following the disappointment of target-selective therapy: instead of picking out a single molecular target we could design an orchestrated attack on multiple sites of the cell's regulatory network to further increase the efficacy of perturbation. But such network-based polypharmacology [8] is still at its infancy and existing quantitative concepts are of a rather ad hoc nature [46]. Whether network approaches can overcome the resilience of tumors afforded by the vast and ubiquitous (non-genetic) heterogeneity of cancer cell populations however remains to be seen.

3. Current interpretations of networks for cancer therapy

A molecular regulatory network can be formally represented as a (mathematical) *graph*: a set of nodes connected by links. Here, we first articulate aspects that have been neglected and point to misconceptions in the interpretation of biomolecular networks that are critical for this discussion.

3.1. Two levels of network analysis: topology and dynamics

Understanding how a biomolecular network governs the fates of a cell requires that we make the fundamental distinction between two levels of analysis which the majority of investigators, with few exceptions [8,13], are not explicitly aware of: network *topology* vs. network *dynamics*. The latter is indispensable for comprehending the integrated function of a network. Unfortunately, many systems biologists not only fail to explicitly make this distinction but they also ignore the very existence of the level of 'dynamics', and thus, they interpret biological function directly from network topology [47]. They view the wiring diagram of the molecular interactions as a graph that represents a network of causations. Arrows are viewed as chains of causation for a phenotype effect: they just need to be disrupted to perturb said phenotype [46] (for more on cancer see Ref. [8] p. 369). For instance, a simple idea is that targeting the "hubs" (highly connected nodes) of a regulatory network will most effectively perturb a cancer cell ("central hit" [8]). Another widely proposed approach is to simultaneously target parallel pathways that impinge on the same regulatory molecule or function thought to be important ("synthetic lethality"), a concept borrowed from genetics [7]. While such intuitively plausible models for disrupting the function of a network can lead to correct predictions [8], the underlying concept is crude and holds only for exceptional scenarios, namely where a linear causal relationship between biomolecule and cell-level function happens to dominate. The counter-intuitive and paradoxical findings regarding drug action, such as lack of effect when one is expected or effects opposite to expectation [48] defy such ad hoc explanations that treat networks as maps of casual relationships.

3.2. Mis-conceptualization of network robustness

One widely used concept is 'robustness' of networks, which in the case of cancer represents the obstacle to be overcome by therapy. But 'robustness' cannot be truly understood without appreciation of network dynamics, for if one stays at the level of network topology, robustness is often simplistically reduced to *redundancy*, the reliance on "back-up connections" (e.g., [49–51] and Refs. herein) when some component have failed – as if the network is a power grid, a high-way or computer network where "something" *flows* through the connections. Accordingly, a perturbation would be understood as the removal (or blocking) of a link or a node that cuts the flow. The central flaw in such thinking is that strictly speaking the links in regulatory networks (but not in metabolic networks) cannot be equated with the familiar physical network connections in which something is passed around. Regulatory networks are not *flow* networks but *influence* networks: the activity of one node *influences* the activity of other nodes connected to it as prescribed by the network architecture.

Thus, targeting strategic nodes as if to disrupt a supply or communication network to disrupts its robust function is a very blunt, albeit sometimes sufficient approach.

3.3. Mis-conceptualization of "function-specific networks"

Another wide-spread misuse of the concept 'network' is the notion that each specific functional cell state has its own 'network' [47]. Accordingly, one speaks of a "stem cell network", "pluripotency network" or identifies a "disease network" that one wishes to disrupt [52]. Such expressions assume that the network topology itself changes: a gene X may regulate gene Y in condition A but not in condition B. Hence, the network is thought to change as cells go from condition A and B. This is a dangerous fallacy for if the very structure of a system is allowed to change according to the behavior produced at a given instance, one abnegates the opportunity to develop a theory that explains how one system (the cell) generates its repertoire of behaviors from within, using a finite set of instructions. The wiring diagram of a TV is the same whether you watch a sport program or a movie. Only if we anchor the description of a system to some *invariant structure* can we begin to understand how the relationships between its (hard-wired) component parts give rise to its wonderful dynamic functionality which is flexible (changing states given appropriate cues) yet robust (to inappropriate signals). To invoke a "stem cell network" [53] to explain the 'stem cell state' or a "disease network" to explain a 'disease state' is, although convenient for some conceptualization and communication, at a deeper level a circular argumentation.

Fig. 2 illustrates that "what changes" when a cell changes its phenotype is the network *state* – not the network topology [47]. The network topology is hard-wired in the genome and captures all *potential* interactions that operate conditioned upon a current network state. This must not be confused with the ad hoc, physical topology of a network of expressed proteins. Thus in this profoundly distinct view there is only one regulatory network per organism (modulo somatic mutations, of course) and it does not change under physiological conditions.

4. Gene network dynamics, attractors

With these caveats we can now discuss network dynamics. We focus for pedagogical simplicity on the gene regulatory network (GRN) where the directed links between the nodes (genes) represent transcriptional regulation of genes. To understand the dynamics of network state S a set of elementary terms must be introduced: the *network state S*, which is a point in the *state space*,

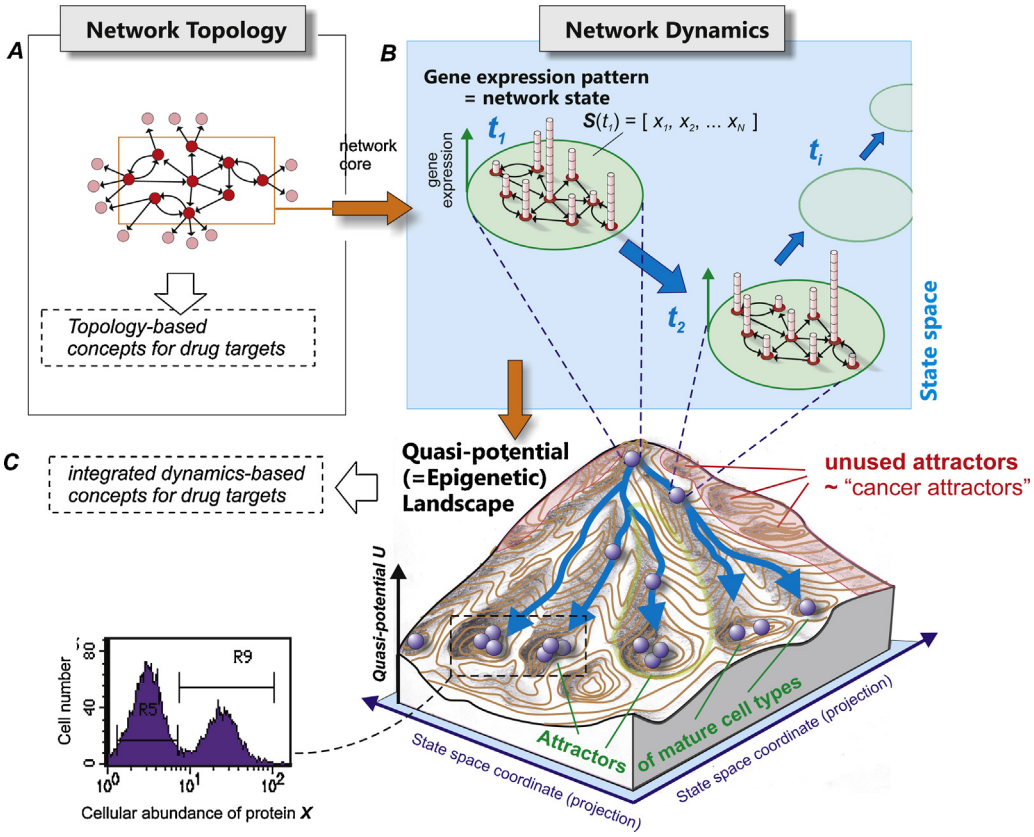

Fig. 2. From gene network topology to network dynamics and the quasi-potential landscape. (*A*) Gene network topology (as in Fig. 1) of *N* genes – only the core (orange box) contributes to the regulation and is considered for the dynamics shown in (*B*). Each green oval represents a gene expression configuration or network state *S*. The entirety of all combinatorically possible gene expression pattern forms the *N*-dimensional state space (schematically shown as blue box). Because of the constraints imposed by the network's regulatory interactions, each network state at time t_1 can only change in a particular way to the "next" one at t_2 etc. (C) Each state *S* in the state space can be assigned a quasi-potential function *U*(*S*), and when the *N*-dimensional state space is projected into a plane, *U*(*S*) can be represented as the elevation over each point *S* in the plane, giving rise to a quasi-potential landscape. Each state is now a marble, rolling down into the valleys along the developmental trajectories ("chreods", blue arrows) leading from the top (embryonic states) to the attractors representing mature cell types. A basin of attraction is highlighted (light green line). Note the attractors in "side valleys" that are not in the developmental paths but in areas never visited during development (dark red shade) – they represent the cancer attractor states. *Inset*: typical flow cytometry measurement showing the quasi-discrete separation of expression level of a protein, manifest in the bimodal population distribution histogram for abundance of a protein marker on each cell (horizontal axis). Such bimodality suggests the presence of attractors.

the vector field and trajectories in it. (For an introductory explanation see Suppl. information and Fig. 2.) In brief, the configuration of the network of *N* gene loci $i = 1,2,\ldots,N$ with respect to their individual activity levels x_i, for $i = 1,2,3,\ldots,N$ defines a state *S* of a GRN at time *t* and is mathematically described by the state vector is $S = [x_1, x_2,\ldots,x_N]$. The network state *S* is a point in the *N*-dimensional state space. A change of state describes a movement of that point in state space along a *trajectory*.

4.1. Stable steady states = attractor states

Such movement of the network in state space that manifests the change of the network state is driven by the regulatory interactions and comes to a stop when all regulatory interactions are "satisfied" – that is, when all the driving "forces" are balanced and vanish. In other words, the network seeks to avoid configurations of gene expression (states) that are not possible (or in a noisy system, very unlikely) in view of the interactions imposed by the network. For instance, the simultaneous high expression of two mutually inhibiting genes *X* and Y (using again the two-gene example, Fig. 3A) must work against what is preordained by the network. As an example, states with the configuration of gene activity values "*X* = *high*" and "*Y* = *high*" are driven to states that have reciprocal expression levels of *X*1 and *X*2, such as "*X* = *low*" and "*Y* = *high*" (or vice versa).

Thus, trajectories of *S* lead from unstable (or in a noisy system: unlikely) states to a steady state ("equilibrium") that is stable and is referred to as an *attractor state* (or simply 'attractors'), so named [54] because in the visual representation of trajectories in state space these stable states appear to 'attract' states in its neighborhood (all or most direction in state space). Because of the attracting property, the attractor states is robust: the network in S^* resists (small) deviations into neighboring states – this is the appropriate, formal notion of "robustness" as opposed the ad hoc meanings encountered above. An attractor state $S^* = [x_1^*, x_2^*,\ldots x_N^*]$ represents "encodes" a specific, stationary, self-stabilizing gene activity configuration of the genome (where each locus *i* is expressed at level x_i^*) which we can now approximately determine by measuring the transcriptome as a surrogate of *S*. Thus, it encodes a particular phenotype (modulo post-translational modification, of course).

4.2. Multi-stability, basins of attraction and unstable states

A particularly interesting feature of complex networks is *multistability* which can arise when the network contains positive feedback loops, which the complex genome-wide network is replete with (e.g., gene *A* stimulates gene *B* which stimulates gene *A;* or a loop of *two* inhibitory interactions as in Fig. 3). Multistability is the coexistence of many attractor states within

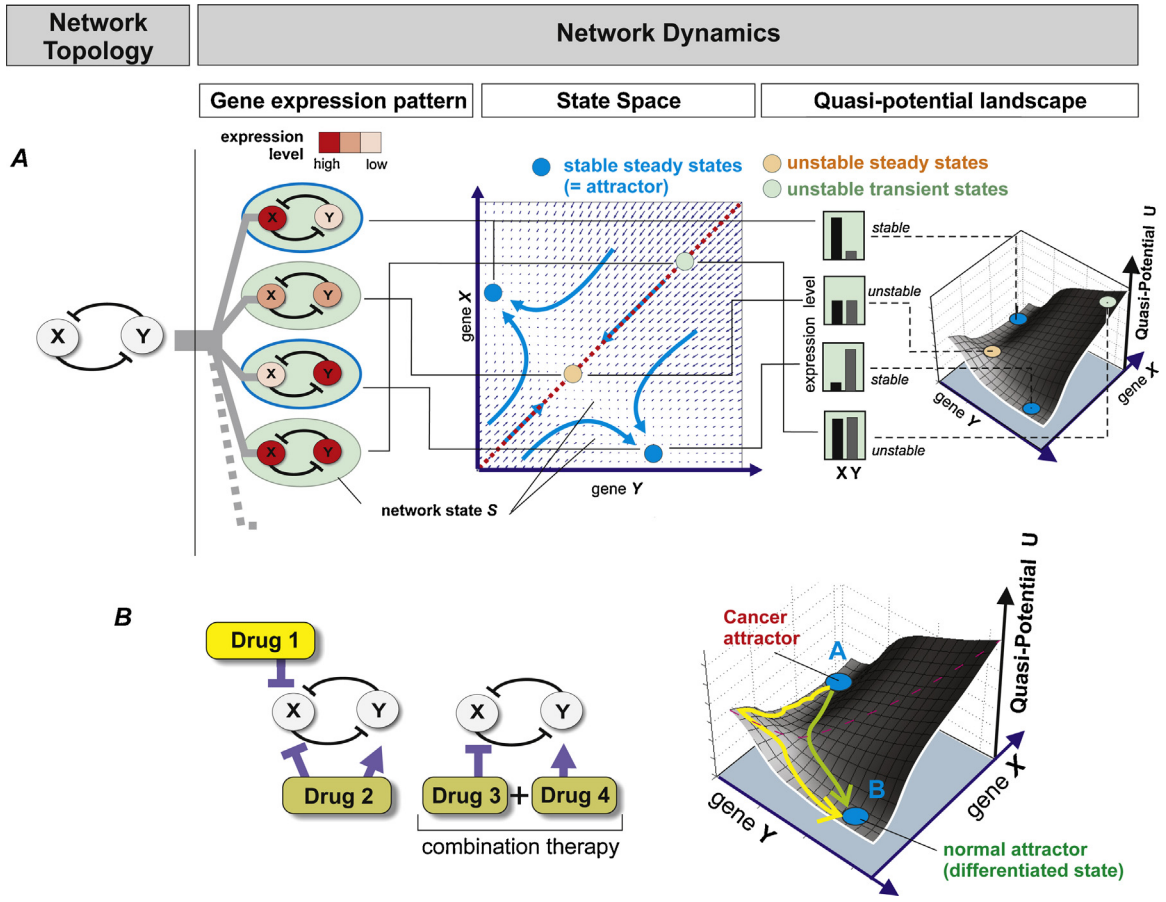

Fig. 3. A two-gene network "toggle switch" model with two attractors illustrating the exit from an attractor via the path of least action. (A) Mapping of gene expression patterns into the state space (as in Fig. 2), now shown as computed vector field (central panel). For details see text. Red dashed line = separatrix (boundary of the two basins of attraction). Large blue arrows indicate overall "flow" toward attractor states. Approximate quasi-potential landscape shown on the right. Note the separation of the two attractor valleys by the "saddle" point (unstable steady state, yellow dot). (B) The advantage of multi-target drugs to cause the exit from attractor *A* (cancer) and go to attractor *B*. The *Drug 1* which only inhibits gene *X* induces changes of the state along the steep yellow path; concomitant activation of *Y* by a multi-target drug, *Drug 2* (or combination therapy; *Drugs 3 + 4*) is equivalent to moving along the green path which is the path of least action.

one network. It is one of the most fundamental properties of complex nonlinear dynamical systems which affords the biosphere the diversity of stable forms. Thus, while negative feedback loops are widely understood to confer stability in the engineering world, positive feedback loops are required to give rise to multistability [55]. The multistable network has multiple phenotypes (attractors) to choose from. In which attractor it ends up depends on the initial state S_0: each attractor state has a domain of attraction, called *basin of attraction*, that contains all the unstable states that will be attracted by the respective attractor state S^*.

4.3. Attractors as cell types

The simple, but formal concept of attractors in networks has long been recognized to be of central importance for biology: attractor states represent cell types – more precisely, the gene expression patterns associated with each cell types, as proposed already by Delbruck [56], Waddington [57], Monod and Jacob [58] in some rudimentary form, and explicitly by Kauffman [59]. Cell types and their transcriptomes are discrete and intrinsically stable phenotypic states: we do not find a continuum of transcriptomes – in line with Waddington's observation that intermediates between two cell types are rare [57]. Such quasi-discreteness is manifest in the readily observable multi-modal (multi-peak) distribution (histogram) of cellular abundance of a cell-type marker protein measured by flow cytometry (inset in Fig. 2C).

4.4. Attractor transitions

If stable cell phenotypes are attractors then any "switch" of phenotype, as occurs in development or disease, are transitions between attractor states [60–63]. The quasi-discreteness of phenotype transitions Fig. 2 (histogram in inset) reflects the transition from one attractor to another. In a simple but permissive view, such transitions can occur when specific signals (hormones, cytokines, drugs) alter the expression of multiple genes (values x_i), such that the position state *S* changes accordingly. A corollary of this principle for understanding multi-target drugs is important and will be discussed later (Section 6): the exit from an attractor typically entails the change of expression x_i of multiple genes in some particular, concerted way. This may be the reason why signal transduction cascades which have evolved to actuate cell phenotype switches in response to cytokines typically fan out to operate a large number of target genes.

Gene expression noise [64,65] also can trigger attractor transitions. The random, often burst-like fluctuations of the activity of locus x_i (or expression levels x_i) when translated into the state space view, appears as a wiggling of the effective path of state *S* along the trajectories or around attractors with less frequent larger

excursions. The latter can cause rare, stochastic spontaneous attractor transitions when the "correct" set of genes (in the appropriate gene-dimensions of the state space) undergo such fluctuations just at the right time and amplitude to mimic the coordinated multigene expression change induced by biological signals to actuate attractor transitions. Such stochastic phenotypic switching of cell fate is readily observed in stem cell fate decision [63,66,67]. These phenomena raise the next important question: *how hard* in general it is to go from one attractor to another which we will discuss in Section 5.

4.5. Cancer attractors

Much as physiological cell types, cancer cells are also discretely distinct and exhibit universally characteristic, "typical" phenotypes which are also self-stabilizing. Thus, they can be viewed as (pathological) cell types in their own right that are not realized in the healthy tissue [detailed argumentation in [23,24]]. Then cancerous states could also be represented by attractor states. This hypothesis was first proposed by Kauffman in 1971 [68]. A refined argument [23] is that during evolutionary growth of the GRN (increase in number of nodes) new attractor states were added and incorporated in the metazoan body as new cell types. But because of mathematical constraints in the mapping from network specification (topology and promoter logics) to the state space structure, a given network contains many more attractors than can be used as cell type. The critical idea is that not every attractor created as the network grew during evolutionary genome expansion can also be readily accessed from the already existing attractors: no developmental path, as explained below, connects existing cell types to these potential (normally not realized) cell phenotypes. It was further proposed [23] that evolution only cares and optimizes the gene expression patterns associated with the attractors that can be accessed because evolutionary selection acts on expressed phenotypes, not on potential phenotypes.

Thus, the unoccupied attractors latently lurk in the dark mathematical space of unexplored regions of the GRN's state space. Attractors here encode immature ("de-differentiated"), un-evolved (or perhaps abandoned after ancient expression), functionally unstable phenotypes. When (re)accessed because of abnormal chronic signals or mutations (see below), the cells that enter them become abnormal. They possess an un-evolved phenotype with unstable cell cycle and low fidelity DNA replication. They can undergo *somatic* evolution and develop into a cancerous state – accessing other unused attractors even further away from the normally used regions of the state space.

If cancer is the departure from the normal cell type attractor, rather than its destruction, then the latter could still be latently present in the network of cancerous cells. This is most impressively exposed by the classical phenomenon of "normalization" of cancer cells: implanting cancer cells in normal tissue environment [69,70] or reprogramming them to pluripotent embryonic cells [71,72] can restore a normal phenotype despite the mutations. In fact the idea behind differentiation therapy [68] is to push these cells back into normal trajectories in state space that lead to differentiation into normal cells, or into apoptosis, senescence, etc. But how does a system (cell) exit [73] a stable attractor state?

5. The quasi-potential landscape and path

The last concept we need to introduce to understand how multitarget perturbation could actuate the switch between attractors, is the quasi-potential landscape which is equivalent to Waddington's metaphoric "epigenetic landscape" that he proposed in the 1950s [74,75]. The landscape concept helps to address the following problem: given the existence of multiple attractor states and the possibility of noise- or perturbation-driven transitions, one wishes to know how "hard" it is to go from one state to another: what is the "relative stability" of the states and the "energy barrier" that separates them? The landscape comes from the notion that a basin of attraction has a *depth* in addition to its size in state space.

5.1. Potential-analog in non-equilibrium systems: the quasi-potential

In classical physics of conserved systems, in which energy is not lost, the relative stability is captured by the energy potential difference ΔU between two states A and B, which can be readily computed directly from the state characteristics and which indicates that the energy expenditure/gain incurred when going from A to B is path-independent. This is not the case for the network states as defined here which are far-from-equilibrium systems [76].

There is no straightforward way to compute such a global potential function. For technical reasons this is not possible because the system equations (see Section 4) that fully describe the driving forces of state change generated by the network interactions are not integrable: we do not have a "gradient system" as in conserved systems: the "energy" needed/released when going from state *A* to state *B* in state space is not path-independent (going in circle is not free of energy expenditure). However, the notion of a "quasi-potential" landscape still can be imagined in the abstract, albeit with many caveats as discussed elsewhere [77]. It is then admissible in this quasi-potential landscape to depict the stable attractor states to valleys, separated by the hills with hilltops representing unstable, poised states and basin boundaries.

5.2. The least action path

Even if the notion of a global potential landscape for GRN is profoundly problematic, one theory permits the precise numerical computation of the difference in a *quasi*-potential $\Delta U(S_A{}^* \rightarrow S_B{}^*)$ between pairs of attractors from the system equations. This transition-specific quantity is, for small noise, directly related to the probability for the attractor transition $S_A{}^* \rightarrow S_B{}^*$. The computation also gives the associated "path of least action" on the quasi-potential landscape [78]. The latter that must be followed to incur the "least action" when going from attractor *A* to *B*, where 'action' can in principle be understood as the 'effort' needed in acting against the attracting force of the attractor state *A* (that the system is exiting from) to an optimal point on the basin-boundary (=separatrix) between the attractors *A* and *B* that needs to be crossed. The attracting forces to be overcome are a consequence of the regulatory constraints imposed by the GRN that produced the attractor in the first place.

Again, let us move from the abstract higher-dimensional systems to our two-dimensional system (Fig. 3) to illustrate the landscape idea. Since here the state space is simply the two-dimensional XY plane, the third (Z-)dimension can be used to display the quasi-potential $U(S)$ of each state *S* as elevation above each point in the *XY*-plane (Fig. 3, right panel) which collectively gives rise to the picture of a landscape with a distinct topography. Then, while down-hill paths to an attractor state are "effort-free" the least action path to exit an attractor will follow the steepest slope to the optimal "saddle point" to exit the old attractor *A* (valley) and enter the new adjacent valley of attractor *B*. For higher dimensional networks, we would have to project the *N*-dimensions of the state space into a two dimensional plane for a mental picture of a potential landscape, as shown in Fig. 2C.

Such landscape pictures must be what Waddington [74,75] intuitively, without notion of gene regulatory networks, had in mind when he proposed the epigenetic landscape to capture the observed

dynamics of cell phenotype change during development. The normal developmental path ("chreod") along which his marble rolls down to reach the lowest point of valleys (stable attractor states) of mature, normal cell types, are equivalent to state space trajectories.

6. Network-based rational therapy: inducing attractor transition

6.1. Cancers are stuck in attractors without connection to chreods

We can now think of the growth of the epigenetic landscape during evolution as a process in which natural selection favors mutations that rewire the network and shapes the landscape topography to render it robust for development. Evolution may have sculpted the landscape such as to shield the developing cells from entering the cancer attractors, keeping them on the trajectories of normal development [75] that guide them to the attractors that encode normal mature cell types whose phenotype has been perfected by evolution to serve the function of the organisms. However, the cancer attractors can be accidentally accessed, when (i) mutations alter the landscape topography (since in our formalism each GRN maps uniquely into a specific landscape) and/or (ii) particular chronic perturbations by non-physiological signals [23,47] shift cell states against large gradients. Then "on their way down" the developing cells so to speak "get stuck" in these cancer attractors that represent abnormal and more immature ("de-differentiated") phenotype with no trajectory available that allows access the normal chreods or attractor states [23].

6.2. Cancer therapy: helping stuck cells to exit cancer attractors

With the concept of the quasi-potential (or epigenetic) landscape and cancer cells stuck in inappropriate attractors in mind it is a small step to comprehend the rationale of a concerted perturbation of the network in cancer treatment: the goal would be to perturb cancer cells such that a transition is triggered out of the cancer attractor side-valley back into a normal chreod that guides the cell to a non-malignant attractor that encodes the differentiation, senescence or apoptosis programs or has easy access to such a state. Differentiation therapy has been proposed by Kauffman along the proposal of cancer attractors [68] and in fact many cancer therapy, notably with non-cytocidal agents, such as interferons, Vitamin D or retinoic acid, do induce a differentiated phenotype [79–81]. However, the problem that such therapy face is that unlike in normal development, no predestined path connects the pathological attractor with the normal regions on the epigenetic landscape.

Thus, we hypothesize the following idealized scheme for therapy: the cancer cells must be pushed, so to speak, through unploughed lands to a normal attractor or at least, a chreod. To do so most efficiently one needs to find the path of least action in the above sense. Once one has computed such a therapeutic path, its initial stretch indicates the direction into which one needs to kick the cell with a drug to most efficiency induce a transition. This approach presents at least in theory a clean, formal rationale for dirty drugs as detailed below. It differs from the ad hoc proposal to hit the strategic nodes of the network most likely to disrupt the cancer cell based solely on network topology considerations.

6.3. Need for multiple drugs to realize the least action path

Let as illustrate this by revisiting the two-gene system with the two attractors, *A* and *B*. One basic assumption is that we have single-target drugs that can suppress or activate one gene (product) at a time, hence: selective drugs move the state only parallel to one axis. As shown in Fig. 3B, assuming one wishes to move from attractor *A* to attractor *B*, it is clear that pushing (through the action of a drug that suppresses *X*) the cell along the *X* axis (yellow paths in Fig. 3B) would enforce a much more uphill climb until one reaches the separatrix to cross over into the basin of attraction *B* than if one chose to suppress *X* and stimulate *Y* simultaneously, equivalent to walking along the diagonal of the state space (green paths in Fig. 3B). In fact, the calculated least action path moves roughly (but not exactly) along the diagonal, leading from attractor *A* to *B*. This implies the need to target *both X* and *Y*. The effort needed to inhibit *X* would be greatly diminished by concomitant stimulation of *Y*. It is clear that in higher-dimensional systems the exit path is even more complex and less likely to project exactly to a state space dimension – hence it will involve the modulation of the activity of all those dimensions to which the exit path has a projection. This is the reason why multi-target modulation is necessary to actuate a switch of attractors. If such a switch of phenotype is the desired manipulation one wishes to achieve, a multi-target or combinatorial approach may be designed such as to operate at least the genes to which the least action path projects substantially.

The need to cross unruly lands with intermediated local attractors that pose obstacles could also be extended to explain the need for specific schemes of sequential treatments [82].

7. Conclusion

While in theory the proposed concepts would allow the identification of the precise set of targets required to cause an attractor switch, we would like to take a broader perspective and recall the reasons for therapy failure listed in Section 2, and in this context point to the following short-comings or our model:

- The prediction of targets depends on the ability to compute the least action path, which implies that one needs the full specification of the network (topology, promoter logics, etc.) Yet, while such detailed knowledge still lies far in the future, the formal framework presented here offers some guidance for designing the meaningful set of targets in terms of qualitative principles (Fig. 3B) beyond what the ad hoc, direct interpretation of gene network topologies offers.
- The discussion here focuses solely on maximizing the ability to switch the cancer (stem) cell toward a benign fate, such as apoptosis or differentiation, and remains in the domain of thought of traditional cancer-cell centered therapy. It does not address promising non-cancer cell targeting modalities such as immunotherapy, anti-inflammation or stromal therapy [83].
- It is not known how far we can go in maximizing the proportion of cells that are effectively targeted. In view of the vast *phenotypic heterogeneity* with respect to responsiveness in a cancer cell population there will be intrinsic limitations to deterministic rationales. The dynamic non-genetic heterogeneity translates into our model as follows: a tumor is not one single marble sitting in its attractor but instead, all the cells of the tumor will be spread over vast stretches within the "cancerous regions" of the quasi-potential landscape around a main cancer attractor. Then, however precise a multi-target perturbation we exert, it will not hit every cell the same way. The sheer diversity of cell states may dwarf even the best tailored perturbation optimized for only one particular state *S*. The cancer attractor emptied by killing the cells in it would then just be replenished with surviving cells from neighboring attractors.

We have little idea of the size and shape of the uncharted region in the epigenetic landscape that all the cells of a tumor occupy because our knowledge of the GRN architecture is limited. Single-cell resolution analysis of gene expression pattern distributions [84–87] of tumor cell populations in the near future will offer a

phenomenological snapshot of the spread of a tumor over the land of cancer attractors. While such a vista may raise the awareness of the daunting challenge of eradicating every single cell hiding in the rugged, normally unvisited wilderness of the epigenetic landscape, it should not discourage us from pursuing a better understanding of network dynamics and cell population dynamics. Even though the coordinated therapy to stimulate exit from the cancerous attractor and entry into a benign attractor using existing or perhaps yet to be discovered sets and sequences of perturbations may work only on a fraction of cancer cells, it may suffice for application in a repeated manner to reduce tumor burden to a manageable chronic disease. The ideas outlined here will be the first step toward a new holistic yet rational and molecular paradigm of therapy in which the current concept of targeting multiple molecules of a network is just one central component.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.semcancer.2013.06.003.

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