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THE THEORY OF BIOLOGICAL ROBUSTNESS AND ITS IMPLICATION TO CANCER

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16.1 INTRODUCTION

Systems biology aims at system-level understanding of biological systems [1,2]. Investigations of biological systems at system level are not a new concept and can be traced back to homeostasis by Walter Cannon, Cybernetics by Norbert Weiner [3], and the general systems theory by von Beltaranffy [4]. Numbers of approaches in physiology have also taken a systemic view of the biological subjects. Systems biology is gaining renewed interest today because of progress in genomics, molecular biology, nonlinear dynamics, computational science, and other related fields.

However, ''system-level understanding'' is a rather vague notion and is often hard to define. This is due to the fact that the system is not a tangible object. Genes and

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proteins are more tangible because they are identifiable matters. Although the system is composed of these matters and they are components of the system, the system itself cannot be made tangible. Often, diagrams of the gene regulatory networks and the protein interaction networks are shown as representations of systems. It is certainly true that such diagrams capture any one aspect of the structures of the system, but they are still only static slices of the system. The heart of the system lies within the dynamics it creates and the logic behind it. It is science on the dynamical state of affairs.

There are four distinct phases that lead us to system-level understanding at various levels. First, system structure identification enables us to understand the structure of the system. While this may be only a static view of the system, it is an essential first step. The structure shall then be identified, ultimately, in both physical and interaction structures. Interaction structures are represented as gene regulatory networks and biochemical networks that indicate how components interact within and in between cells. Physical details of specific regions of the cell, overall structure of cells, and organisms are also important because such physical structure imposes constraints on possible interactions and the outcome of interactions impacts the formation of physical structures. Nature of interaction could be different if proteins involved in interaction move by simple diffusion or under specific guidance from cytoskeleton.

Second, system dynamics needs to be understood. Understanding the dynamics of the system is an essential aspect of the study in systems biology. This requires integrative efforts of experiments, measurement technology development, computational model development, and theoretical analysis. Various methods, such as bifurcation analysis, have been used, but further investigations are necessary to handle the dynamics of systems with very high dimensional space.

Third, methods to control the system shall be investigated. One of the implications is to find a therapeutic approach based on system-level understanding. Many drugs have been developed through extensive effect-oriented screening. It is only recently that specific molecular targets have been identified and lead compounds are designed accordingly. Success in control methods of cellular dynamics may enable us to exploit intrinsic dynamics of the cell so that its effects can be precisely predicted and controlled.

Finally, designing the system that is to modify and construct biological system with designed features. Bacteria and yeast may be redesigned to yield desired properties for drug production and alcohol production. Artificially created gene regulatory logic could be introduced and linked to innate genetic circuits to attain desired functions [5].

Several different approaches can be taken within systems biology field. One may decide to carry out a large-scale, high-throughput experiment and try to find out the overall picture of the system at coarse-grain resolution [6–9]. Alternatively, working on precise details of specific signal transduction [10,11], cell cycle [12,13], and other biological issues to find out the logic behind them are a viable research approach. Both approaches are essentially complementary, and, together, can reshape our understanding of biological systems.

16.2 ROBUSTNESS IS THE FUNDAMENTAL ORGANIZATIONAL PRINCIPLE OF BIOLOGICAL SYSTEMS

Robustness is a property of the system that maintains a certain function despite external and internal perturbations that are ubiquitously observed in various aspects of biological systems [14]. It is distinctively a system-level property that cannot be observed by just looking at components. Specific aspects of the system, the functions to be maintained, and the types of perturbations that the system is robust against must be well defined to make solid arguments. For example, a modern airplane (system) has a function to maintain its flight path (function) against atmospheric turbulences (perturbations).

Bacteria chemotaxis is one of the most well-documented examples in which chemotaxis is a function maintained against the perturbations that are changes in ligand concentration and rate constants for the interactions involved [15–17]. The network for segmental polarity formation during *Drosophila* embryogenesis robustly produces repetitive stripes of differential gene expressions despite variations in initial concentration of substances involved, as well as kinetic parameters of interactions [18,19]. Various aspects of robustness of biological systems have been studied extensively, but more remains to be explored and formalized to create solid theoretical foundations.

Why is robustness so important? First of all, it is a feature that is observed to be so ubiquitous in biological systems; from such a fundamental process like phage fate decision switch [20] and bacteria chemotaxis [15–17] to developmental plasticity [18] and tumor resistance against therapies [21,22]. This implies that it may be a basis for principles that are universal in biological systems, as well as being opportunistic toward finding cures for cancer and other complicated diseases.

Second, robustness is a system-level property of the system in which interactions of components give rise to this feature. Robustness in this context refers to a feature of the system to maintain its function instead of structures or specific states. Structures or states can be dynamically changed if they lead to maintenance of the function of the system.

Third, robustness against environmental and genetic perturbation is essential for evolvability [23–25]. Evolvability requires generation of variety of nonlethal phenotype and genetic buffering [26,27]. Mechanisms that attain robustness against environmental perturbation may be used also for attaining robustness against mutations, developmental stability, and other features that facilitate evolvability [14,23–25].

Fourth, it is one of the features that distinguish biological systems and man-made engineering systems. Although some man-made systems, such as airplanes, are designed to be robust against the range of perturbations, most man-made systems are not as robust as biological systems. Some engineering systems that are designed to be highly robust entail mechanisms that are also present in life forms, which imply existence of the universal principle.

16.3 UNDERLYING MECHANISMS FOR ROBUSTNESS

16.3.1 System Control

First, extensive systems control is used, mostly saliently negative feedback loops but also feedforward and positive feedback controls, to make a system dynamically stable around the specific state of the system. An integral feedback is used in bacteria with chemotaxis as a typical example [15–17]. Due to integral feedback, bacteria can sense changes of chemoattractant and chemorepellant independent of absolute concentration so that proper chemotaxis behavior is maintained over a wide range of ligand concentration. In addition, the same mechanism makes it insensitive to changes in rate constants involved in the circuit. Positive feedbacks are often used to create bistability in signal transduction and cell cycle, so that the system is tolerant to minor perturbation in the stimuli [10,12,13].

16.3.2 Fault Tolerance (Redundancy and Diversity)

Second, fault tolerance mechanisms increase tolerance against components failure and environmental changes by providing alternative components or methods to ultimately maintain a function of the system. Sometimes there are multiple components that are similar to each other and are redundant. Other cases are different means that they are used to cope with perturbations that cannot be handled by the other means. This is often called phenotypic plasticity [28,29] or diversity. Redundancy and phenotypic plasticity are often considered as opposite things, but it is more consistent to view them as different ways to meet an alternative fail-safe mechanism.

16.3.3 Modularity

Third, modularity provides isolation of perturbation from the rest of the system. The cell is the most significant example. More subtle and less obvious examples are modules of biochemical and gene regulatory networks. Modules also play an important role during developmental processes that buffer perturbations so that proper pattern formation can be accomplished [18,30,31]. The definition of the module and the methods of how to detect such modules are still controversial, but the general consensus is that the module does exist and play an important role [32].

16.3.4 Decoupling (Buffering)

Fourth, decoupling isolates low-level noise and fluctuations from functional-level structures and dynamics. One example here is genetic buffering by Hsp90 in which misfolding of proteins due to environmental stresses is fixed, and thus effects of such perturbations are isolated from the functions of the circuits. This mechanism also applies to genetic variations where genetic changes in coding region that may affect protein structures are masked because protein folding is fixed by Hsp90, unless such masking is removed by extreme stress [24,33,34]. Emergent behaviors of complex

networks also exhibit such buffering properties [35]. These effects may constitute canalization proposed by Waddington [36]. A recent discovery by Uri Alon's group on oscillatory expression of p53 upon DNA damage may exemplify decoupling at signal-encoding level [37], because stimuli invoked pulses of p53 activation level, instead of gradual changes, effectively converting analogue into digital signal. Digital pulse encoding may indicate robust information transmission, although further investigations are clearly warranted to draw any conclusion at this moment.

An example of a sophisticated engineering system clearly illustrates how these mechanisms work as a whole system. An airplane is supposed to maintain a flight path following the command of the pilot against atmospheric perturbations and various internal perturbations, including changes in the center of gravity due to fuel consumption and movement of passengers, as well as mechanical inaccuracies. This function is carried out by controlling flight control surfaces (rudder, flaps, elevators, etc.) and a propulsion system (engines) by an automatic flight control system (AFCS). Extensive negative feedback control is used to correct deviations of flight path. The reliability of the AFCS is critically important for stable flight. To increase reliability, the AFCS is composed of three independently implemented modules (a triple redundancy system) all of which meet the same functional specification. Most parts of the AFCS are digitalized, so that low-level noise of voltage fluctuations is effectively decoupled from digital signals that define the function of the system. Due to these mechanisms, modern airplanes are highly robust against various perturbations.

16.4 INTRINSIC FEATURES OF ROBUST SYSTEMS: EVOLVABILITY AND TRADE-OFFS

For the system to be evolvable, it must be able to produce variety of nonlethal phenotypes [27]. At the same time, genetic variations need to be accumulated as a neutral network so that pools of genetic variants are exposed when the environment suddenly changes. Systems that are robust against environmental perturbations entail mechanisms such as system control, alternative, modularity, and decoupling that also support, by congruence, generation of nonlethal phenotype and genetic buffering. In addition, the capability to generate flexible phenotype and robustness requires the emergence of the bow tie structure as an architectural motif [38]. One of the reasons why robustness in biological systems is so ubiquitous is that it facilitates evolution, and evolution tends to select traits that are robust against environmental perturbations. This leads to successive addition of system controls.

Systems that acquire robustness against certain perturbations through design or evolution have intrinsic trade-offs between robustness, fragility, performance, and resource demands. Carlson and Doyle argued, using simple examples from physics and forest fire, that systems that are optimized for specific perturbations are extremely fragile against unexpected perturbations [39,40]. A system that has been designed, or evolved, optimally (either globally optimal or suboptimal) against certain perturbations is called a high optimized tolerance (HOT) system. Ceste and Doyle further

argued that robustness is a conserved quantity [41]. This means when robustness is enhanced against a range of perturbations, it must then be paid off by fragility elsewhere as well as compromised performance and increased resource demands.

Robust-yet-fragile trade-offs can be understood intuitively using the airplane example yet again. When comparing modern commercial airplanes with the Wright Flyer, modern commercial airplanes are, by a great magnitude, more robust against atmospheric perturbations than the Wright flyer, and are thus attributed to a sophisticated flight control system. However, such a flight control system fully relies on electricity. In a very unthinkable event of total power failure in which all electricity is lost in the airplane, the airplane cannot be controlled at all. Obviously, airplane manufacturers are well aware of this issue and take all possible counter measures to minimize such a risk. On the other hand, despite its vulnerability against atmospheric perturbations, the Wright flyer will never be affected by the power failure because there is no reliance on electricity. This extreme example illustrates that systems that are optimized for certain perturbations could be extremely fragile against unusual perturbations.

HOT model systems are successively optimized/designed (not necessarily globally optimized, though) against perturbations in contrast to self-organized criticality (SOC) [42] or scale-free networks [43] that are unconstrained stochastic additions of components without design or optimization involved. Such differences actually affect failure patterns of the system, and thus have direct implications on understanding the nature of disease and therapy design.

Unlike scale-free networks, HOT systems are robust against perturbations like removal of hubs as far as systems are optimized against such perturbations. However, systems are generally fragile against ''Fail-on'' type failure in which components failure results in continuous malfunction, instead of cease to function ''Fail-off,'' so that incorrect signals are kept transmitted. This type of failure is known in the engineering field as the Byzantine Generals Problem [44], named after the problem in the Byzantine army composed of numbers of generals dispersed in the field, some of them traitors who sent incorrect messages to confuse the army.

Disease often reflects the systemic failure of the system triggered by the fragility of the system. Diabetes mellitus is an excellent example of how systems that are optimized for near-starving, intermittent food supply, high energy utilization lifestyle, and highly infectious conditions are fragile against unusual perturbations such as high energy containing foods, and a low energy utilization lifestyle [45]. Due to optimization toward a near-starving condition, the extensive control to maintain a minimum blood glucose level is acquired so that activities of central neural systems and innate immunity are maintained. However, no effective regulatory loop has been developed against excessive energy intake and feedback regulations work to reduce glucose uptake by adipocyte and skeletal muscle cells because it may reduce plasma glucose level below the acceptable level. These mechanisms lead to a state where blood glucose level is chronically maintained higher than the desired level, from the longer time scale that has not been optimized for, further leading to cardiovascular complications. Similar observations have been made for autoimmune disorders where the

evolution of robust immunity also entails proinflammatory and hyperactive immune system [46].

16.5 SELF-EXTENDING SYMBIOSIS

So far, robustness and its relationship with evolution have been argued within the framework of Mendel's genetics in a sense that mutation and crossover through mating has been considered as a mechanism for evolutionary innovations. Emergence of specific mechanisms for increasing robustness and enrichment of bow tie structure has been discussed within this paradigm. I have previously proposed that there may be other means of enhancing robustness through evolution, but by extending ''self'' with foreign biologic substances, a notation that I termed ''self-extending symbiosis'' [47]. Self-extending symbiosis is a phenomenon where evolvable robust systems continue to extend their system boundary by incorporating foreign biologic forms (genes, microorganisms, etc.) to enhance their adaptive capability against environmental perturbations, hence improving their survivability and reproduction potential. In other words, robust evolvable systems have consistently extended themselves by incorporating nonself into tightly coupled symbiotic states.

Looking at the history of evolutionary innovations, it has become clear that some of the major innovations are the result of acquisition of ''nonself'' into ''self'' at various levels. Horizontal gene transfer (HGT) facilitates evolution by exchanging genes of different species that have evolved for different optimization contexts, and was shown to be a frequently observed phenomenon in prokaryotes, archea, and unicellular eukaryotes [48,49]. Microorganisms acquire novel functions, mostly to enhance their robustness against environmental challenges, through horizontal exchange of genes. For example, it has been argued that global emergence of antibiotic- resistant bacteria may be caused by horizontal transfer of antibiotic genes [50–52]. In metazoan species, HGT has not been reported (at best, reported highly controversially) except in some rare instances on insect–bacteria symbiosis between the adzuki bean beetle *Callosobruchus chinensis* and *Wolachia* [53].

The serial endosymbiosis theory by Lynn Margulis [54,55] argues that eukaryotic cells have been created by acquiring bacteria as their organelles. This resulted in greater functionalities of eukaryotic cells, hence more robust against environmental challenges. Here, symbiosis resulted in incorporation of foreign biologic entity into cytoplasm as well as into its own genome.

While HGT and endosymbiosis resulted in incorporation of foreign biologic entity into genome and cellular structure, there are forms of symbiosis that do not directly alter genome but essential to the survival of the species. There are species that allow certain bacteria to be vertically inherited through the host's oocytes as observed in sponges, clams [56], and aphids [57]. Aphids, for example, are infected with the genus *Buchnera*, resulting in an endosymbiotic relationship and acquired dramatically improved energy utilization and terrain exploration capability. It was shown that aphids and *buchnera* undergo parallel evolution where the phylogeny trees of the host (aphids) and symbionts (genus *Buchnera*) are consistent [57]. A case

of parallel evolution has also been observed in endosymbiosis of *Psyllid* and *Candidatus* [58].

Apart from such tight coupling of host and symbiont, horizontal (environmental) acquisition of symbionts [59] is yet another approach in extending the self by incorporating a broader range of microbes, thereby allowing the host to be able to adapt to a broader range of environments and nutrients. Commensal bacterial flora are ubiquitously observed in various metazoan species, including termites [60], cockroaches [61], prawns [62], and mammalians, and have established inseparable relationships with the host organisms, and are even considered to have coevolved [63]. In human beings, the commensal bacterial flora in the gut consists of diverse microorganisms up to 500–1000 species, amounting to about 10^{14} bacteria weighing a total of 1.5 kg [64]. The human being as a symbiotic system consists of approximately 90 percent prokaryotes and 10 percent eukaryotes [65], and a random shotgun sequencing of the whole human symbiotic system would result in predominantly bacterial genome readouts of about 2 million genes with sporadic mammalian genes [66]. Such commensal intestinal bacteria play a critical role in various aspects of the host physiology. Mammalian bacterial flora has been considered to constitute an integral part of host protection by mutually beneficial symbiosis with the host immune system.

The line of observations point to the characteristic property of biological systems that the greater levels of robustness and functionalities is gained by incorporating foreign biologic entities into their own system in the form of different degree of symbiosis. HGT and endosymbiosis incorporate foreign entities into genome and cellular structures, where vertical inheritance based endosymbiosis do not directly alter the genome. Bacterial flora simply adds a layer of adaptive system that is symbiotically interacting with mucosal immune system of the host. A general tendency observed here is the continuous addition of external layers by symbiotic incorporation of foreign entities, and increased level of robustness against environmental perturbation is gained in this process.

16.6 CANCER AS A ROBUST SYSTEM

Cancer is a heterogeneous and highly robust disease that represents worse case scenario of system failure; a fail-on fault where malfunction components are protected by mechanisms that support robustness in normal physiology [21,22]. It is a robustness hijack. Survival and proliferation capability of tumor cells are robustly maintained against a range of therapies due to intratumoral genetic diversity, feedback loops for multidrug resistance, tumor–host interactions, and so on.

Intratumoral genetic heterogeneity is a major source of robustness in cancer cells. Chromosome instability facilitates generation of intratumoral genetic heterogeneity through gene amplification, chromosomal translocation, point mutations, aneuploidy, and so on [67–70]. Intratumoral genetic heterogeneity is one of the most important features of cancer that provides alternative, or fail-safe mechanisms for tumor to survive and grow again despite various therapies, because some tumor cells may have

genetic profile that are resistant to the therapies carried out. Although there are only a few studies on intratumoral genetic heterogeneity, available observations in certain types of solid tumors indicate that there are multiple subclusters of tumor cells within one tumor cluster in which each subcluster has different chromosomal aberrations [71–75]. This implies that each subcluster is developed as clonal expansion of a single mutant cell, and creation of a new subcluster depends upon the emergence of a new mutant that is viable for clonal expansion. A computational study demonstrates that spatial distribution within a tumor cluster enables the coexistence of multiple subclusters [76].

Multidrug resistance is a cellular-level mechanism that provides robustness of viable tumor cell against toxic anticancer drugs. In general, this mechanism involves overexpression of genes such as MDR1 that encodes ATP-dependent efflux pump, P-glycoprotein (P-gp) that effectively pumps out broad range of cytotoxins [77,78]. Trials to mitigate function of P-gp using verapamil, cyclosporine its derivative PSC833 have been disappointing [79].

Tumor–host interactions play major roles in tumor growth and metastasis [80]. When tumor growth is not balanced by vascular growth, hypoxic condition emerges in a tumor cluster [81]. This triggers HIF-1 upregulation that induces a series of reactions that normally function to maintain normal physiological conditions [82]. Upregulation of HIF-1 induces upregulation of VEGF that facilitates angiogenesis, and uPAR and other genes that enhance cell motility [81]. These responses solve hypoxia of tumor cells either by providing oxygen to tumor cluster or by moving tumor cells to a new environment—resulting in further tumor growth or metastasis. Interestingly, macrophages are found to chemotaxis into tumor cluster. Such macrophages are called tumor-associated macrophage (TAM), and found to overexpress HIF-1[83]. This means that the macrophage that is supposed to remove tumor cells may be built-in to feedback loops to facilitate tumor growth and metastasis.

In addition, it can be considered that tumor cells may evolve through self-extending symbiosis. If this is the case, tumor cells shall enhance their robustness against various perturbations through horizontal gene transfer, symbiosis with other cells in the form of cell fusion, and formation of symbiotic relationship with surrounding environments. Interestingly, recent reports indicate that tumor cells may be actively involved in cell fusion and uptake of chromosomes of other cells [84–87]. In addition, artificially produced hybrodimas between antibody-producing plasma cell and tumor cell are used for monoclonal antibody production indicating stable maintenance of cellular function upon hybridization. These series of observations imply that tumor cells may be considered as a group of cells that have become somewhat detached from the host system and have begun evolving independently, so that a wide range of phenomena, such as self-extending symbiosis, also occur on tumor cells and thereby their robustness against perturbation is enhanced (Fig. 16-1).

So far, such phenomena have only been reported independently, and not been placed in the perspective. Reorganizing these findings under the coherent view of cancer robustness will provide us a guideline for further research.

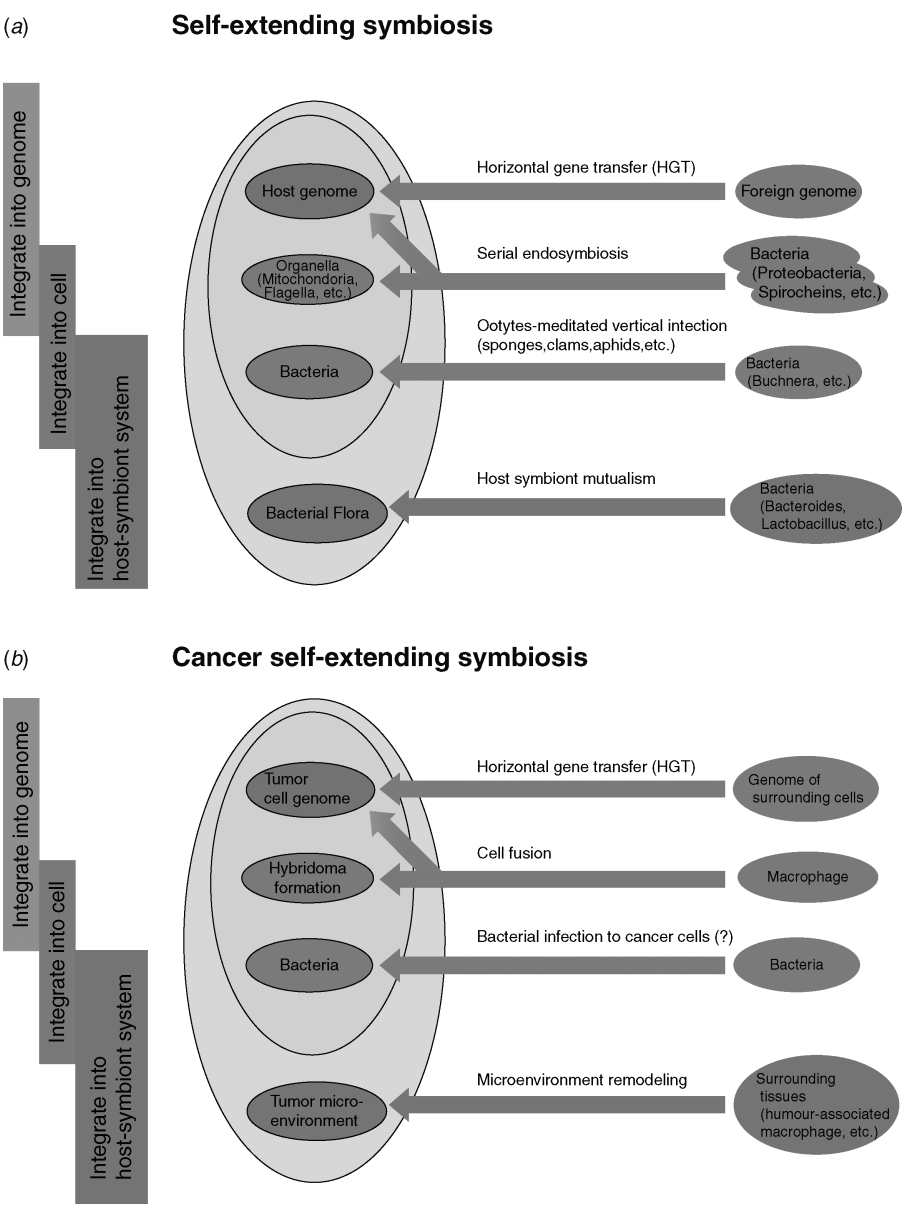

Figure 16-1 Self-extending symbiosis and cancer evolution. Self-extending symbiosis is a path that multicellular organism might have gone through in the course of evolution. Acquisition of nonself into self at various levels of flexibility enhances robustness of organisms against various perturbations. Cancer may also evolve through self-extending symbiosis. Assume cancer as an independent species diverted from somatic cell, it may rapidly evolve through bacteria-like horizontal gene transfer, cell fusion, and microenvironment remodeling to enhance robustness against environmental perturbation. In self-extending symbiosis, there is clear evidence of ootytes-mediated vertical infection. There is no conclusive report if any bacterial infection is observed in any type of cancer that affects robustness of cancer against perturbation. Such phenomena might have been simply unnoticed waiting for future discovery.

16.7 THEORETICALLY MOTIVATED THERAPY STRATEGIES

Given the highly complex control and heterogeneity of tumor, random trial of potential targets is not as effective as one wish it to be. There is a need for theoretically motivated approach that guides us to identify a set of therapies to best counter the disease. The implication of the theory of cancer robustness is that there are specific patterns of behaviors and weakness in robust systems as well as rational way of controlling and fixing the system, and such general principles also apply to cancer. Thus, there must be theoretically motivated approach for the prevention and treatment of cancer. This section discusses therapeutic implications of the theory.

Strategy for cancer therapy may depend upon the level of robustness that the tumor of a specific patient has. When robustness is low, and genetic heterogeneity is low, then there is a good chance that the use of drugs with specific molecular targets may effectively cure cancer by causing the common mode failure: a type of failure in which all redundant subsystems fail for the same reason. An example of CML (chromic myeloid leukemia) therapy by imatinib metylate (Glivec: Novertis) may provide us some insights [88,89]. Dramatic effects of imatinib metylate for early stage CML stem from the fact that it selectively target BCR-ABL protein that is specifically expressed in tumor cells and tumor growth depends on BCR-ABL [90]. Thus, it causes the common mode failure in tumor cells that have similar fragility. However, it is resistant in advanced stage due to heterogeneity of mutations so that the drug cannot inhibit diverse emergent mutant proteins [91]. For this strategy to be effective, there must be proper means to diagnose the degree of intratumoral genetic variations. Then, the most effective molecule as a target needs to be recognized that directs the lead identification and optimization processes.

However, for patients with an advanced stage cancer, intratumoral genetic heterogeneity may be already high and various feedback controls may be significantly upregulated. In these cases, drugs that are effective in the early stage may not work as expected, due to heterogeneous response of tumor cells and feedbacks to compensate for perturbations. For these cases, therapy and drug design need a drastic shift from molecule-oriented approach to a system-oriented approach. Then, the question is which approach shall be taken to target the system, instead of the molecule. I would consider that there are three theoretically motivated countermeasures.

First, robustness/fragility trade-off implies that the cancer cells that have gained increased robustness against various therapies may have a point of extreme fragility. Targeting such a point of fragility may bring dramatic effects for the disease. The major challenge is to find such a point of fragility. Since this trade-off emerged due to successive modifications of the system design to optimally cope with specific perturbations, it is essential to identify the perturbations that the system is optimized against and the underlying mechanisms that enable such optimization. For example, one mechanism for tumor robustness is enhanced genetic heterogeneity that is generated by chromosomal instability, so that some cells may have genetic profile suitable for survival under the specific pressure from the therapy. Then, a method to enhance chromosomal instability selectively in cells that already have unstable chromosome could be one candidate. The point here is whether such

effects can be done with sufficient selectivity. Nonselective approach to increase chromosomal instability has been proposed [92], but it may enhance chromosome instability of the cells that are relatively stable now and thus potentially promotes malignancy.

Second, approaches that avoid the increase of robustness constitute the other possibility. Since genetic heterogeneity is enhanced, at least in part, by somatic recombination, selectively inducing cell cycle arrest to tumor cells can effectively control the robustness. There is a theoretical possibility that such subtle control can be done by careful combination of multiple drugs that specifically perturb biochemical interactions. A computational study indicates that the removal or attenuation of specific feedback loops involved in cell cycle reduces the robustness of cell cycle against changes in rate constant [93]. The challenge is to find appropriate combination of drugs that can effectively induce cell cycle arrest only in tumor cells, but not in other cells. Although this approach uses combination of multiple drugs, there is hope to find a set of drugs that can be administered at minimum dosage and toxicity. This approach results in the dormancy of the tumor. Cancer dormancy has already been proposed [94,95] and many report that induced dormancy has been found in mouse [96,97]. However, these studies report cases where tumor cell proliferation is offset by increased apoptosis. Since heterogeneity may increase by cell proliferation, this type of dormancy, which I call ''pseudo dormancy'' does not prevent increase in heterogeneity, hence robustness is not controlled. Genuine dormancy needs to induce selective cell cycle arrest.

Third, an approach to actively reduce intratumoral genetic heterogeneity followed by a therapy by molecular targeted drugs may be a viable option. If we can design an initial therapy to impose a specific selection pressure on the tumor in which there are only cells with specific genetic variations to survive the therapy, then reduction of genetic heterogeneity may be achieved. Then, if a tumor cell population is sufficiently homogeneous, a drug that specifically targets a certain molecule may have significant impact on the remaining tumor cell population. An important point here is that the drugs used shall not enhance mutation and chromosomal instability. If mutations and chromosomal instability are enhanced, particularly by the initial therapy, heterogeneity may quickly increase so that the second line therapy will be ineffective.

Fourth, one may wish to retake control of the feedback loops that give rise to robustness in an epidemic state. Since the robustness of tumor is often caused by host tumor feedback controls, robustness of tumor can be seriously mitigated if such feedback loops can be controlled. One possible approach is to introduce a decoy that effectively disrupts feedback control or invasive mechanisms of the epidemic. Such an approach is proposed in AIDS therapy where conditionally replicating HIV-1 (crHIV-1) vector that has only *cis* region but no *trans* is introduced [98,99]. This decoy virus dominates the replication machinery, so that HIV-1 virus is pushed into latency, instead of eradication. In solid tumor, an interesting idea has been expressed to use TAM as delivery vehicle of the vector [83,100]. TAM migrates into solid tumor cluster and upregulates HIF-1 that facilitates angiogenesis and metastasis. If TAM can be used to retake a control, robustness may be well controlled and self-extending symbiosis in cancer evolution may be aborted.

Finally, multicomponent drugs may be designed where each component targets molecule in which perturbation differentially affects tumor and normal cells. A certain perturbation affects more the tumor cells but less the normal cells. Even if each of such perturbations does not eliminate tumor cells or is not able to stop their proliferation, there may be specific combination of such drugs that in synergy affects drastically and selectively the tumor cells. One extreme of such approach is the ''long-tail drug,'' recently proposed by the author, that uses large numbers of weakly interacting compounds to affect the tumor cells [101].

16.8 A PROPER INDEX OF TREATMENT EFFICACY

It is important to recognize that, in the light of cancer robustness theory, tumor mass reduction is not an appropriate index for therapy and drug efficacy judgment. As discussed already, reduction of tumor mass does not mean that proliferation potential of tumor has generally decreased. It merely means that subpopulation of tumor cells that are respondent of the therapy were eradicated, or significantly reduced. The problem is that the remaining tumor cells may be more malignant and aggressive, so that therapies for relapsed tumor could be extremely ineffective. This is particularly the case, drugs used to reduce tumor mass are toxic and potentially promote mutations and chromosomal instability in nonspecific ways. It may even enhance malignancy but imposing selective pressures to select resistant phenotype, enhance genetic diversity, as well as providing niche for growth by eradicating fragile subpopulation of tumor cells.

The proper index shall be based on control of robustness: either minimizes the increase of robustness or reduces robustness. This can be achieved by inducing dormancy, actively imposing selective pressure to reduce heterogeneity or exposing fragility that can be the target of therapies to follow, and retaking control of the feedback regulations. The outcome of controlling the robustness may vary from moderate growth of tumor, dormancy that is no tumor mass growth or significant reduction in tumor mass. It should be noted that robustness control does not exclude the possibility of significant tumor mass reduction. If we can target a point of fragility of tumor, it may trigger a common mode failure and may result in significant tumor mass reduction. However, this is a result of controlling robustness, and should not be confused as therapy aimed that tumor mass reduction because robustness has to be controlled to the first to actively exploit a point of fragility. Except for the fragility attack, other options seek for dormancy that results in no tumor growth.

However, this criterion poses a problem for drug design, because current efficacy index of antitumor drugs is measured on the basis of tumor mass reduction. Drugs that induce dormancy will not satisfy this efficacy criterion; thus, they are most likely to be rejected in Phase-II stage. On the other hand, this means that many compounds that have been rejected in Phase-II could be effective from the point of robustness control. Whether such approach can be taken depends on perception change in practitioners, drug industries, and regulatory authorities.

16.9 CONCLUSION

This chapter discussed basic ideas behind the theory of biological robustness and its implications for cancer research and treatment. Biological robustness is one of the essential features of living systems that argued to be tightly coupled with evolution. It may also shape the basic architectural feature of biological systems that are robust and evolvable. One of major consequences is trade-offs between robustness, fragility, resource demands, and performance. Fragility is particularly relevant to diseases. At the same time, cancer established its own robustness. It may be the result of hijacking the robustness intrinsic to the host system. Understanding of this complex nature of biological systems may have profound implications for biomedical research in future.

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