Gene Regulation and Systems Biology

PERSPECTIVE

Implications of Systemic Dysfunction for the Etiology of Malignancy

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Abstract: The current approach to treatment in oncology is to replace the generally cytotoxic chemotherapies with pharmaceutical treatment which inactivates specific molecular targets associated with cancer development and progression. The goal is to limit cellular damage to pathways perceived to be directly responsible for the malignancy. Its underlying assumptions are twofold: (1) that individual pathways are the cause of malignancy; and (2) that the treatment objective should be destruction—either of the tumor or the dysfunctional pathway. However, the extent to which data actually support these assumptions has not been directly addressed. Accumulating evidence suggests that systemic dysfunction precedes the disruption of specific genetic/molecular pathways in most adult cancers and that targeted treatments such as kinase inhibitors may successfully treat one pathway while generating unintended changes to other, non-targeted pathways. This article discusses (1) the systemic basis of malignancy; (2) better profiling of pre-cancerous biomarkers associated with elevated risk so that preventive lifestyle modifications can be instituted early to revert high-risk epigenetic changes before tumors develop; (3) a treatment emphasis in early stage tumors that would target the restoration of systemic balance by strengthening the body's innate defense mechanisms; and (4) establishing better quantitative models of systems to capture adequate complexity for predictability at all stages of tumor progression.

Keywords: targeted therapy, complex systems, quantitative modeling, tumor microenvironment

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Cancer and Complexity

A tumor's ability to adapt

Following the success of Gleevec in treating chronic myelogenous leukemia (CML) by disrupting the signal from the BCR-ABL kinase, a large number of small molecule therapeutics (SMTs) targeting oncogenes entered development. A number of these have now reached the clinic after successful trials, however, in all cases excluding CML, resistance of cancer cells to treatment appears relatively rapidly (typically on the time scale of months). Despite careful study of tumor biology and initial positive responses to therapy, cancers appear to have a virtually limitless ability to respond to treatments by reprogramming cells and developing resistance to specific therapeutics.

Cells in general and cancer cells in particular are complex, highly nonlinear systems. The primary assumption underlying the targeted treatment approach is that the cause of specific types of cancer can be reduced to a singularity, e.g., an aberrant gene or signaling pathway that should be the treatment target. However, increasing evidence suggests that the mutations and dysfunctional signaling pathways dominant in most types of tumors may be occurring as a result of prior dysfunction in multiple other systems.[1–6] This perspective suggests that cancer begins as a systemic malfunction that enables tumorigenesis at a localized site. If this is correct, and accumulating data support this view, then cancer would more appropriately be seen as a complex, systemic disease[1,7,8] and difficulties with the current pharmacologic approach might be explained.

Complex diseases are characterized not only by multiple genetic and environmental contributors but also by emergent properties, which are a result of the underlying nonlinearity and fractal structure of many systems (e.g., his-purkinje fibers in the heart, bronchi in the lungs). Emergent properties are characteristics or behaviors that arise when a group of molecules or entities merge/interact to form a new entity (e.g., a protein, cell, or organ) with novel properties that were unpredictable from the individual components. They are entirely new characteristics that do not exist in the original components. Because they are not the sum of their constituent parts, reductionist methods are of little use in understanding or predicting them. The most well-known example of an emergent property is consciousness, which arises from the interactions of the neurons of the brain. It is impossible to predict that the complex neural network driven by electrical and biochemical changes can generate a system capable of contemplating the actions of its own constituent parts. Likewise, the cell signaling networks underlying cell behaviors and their coupling to biochemical signals from neighboring and distant cells create a similarly complex set of interactions.[9] Therefore we expect emergent properties in this system as well, and we propose here that cancer is potentially one such emergent property.

Error tolerance in gene and molecular networks

Signaling networks within cells and between cells are part of the infrastructure that contributes to emergent properties.[9] The receptors, genes, cells, and signaling pathways that have been individual targets for pharmacologic intervention are all part of dynamic, interconnected networks whose structures are not random.[10] Some of these gene networks, such as developmental networks, are very strictly regulated and therefore resistant to change;[10] while others are variable and controlled by multiple regulatory elements responding to intra- and extracellular cues. The ca. 20,000 protein coding genes in the human genome interact with networks of transcription factors and other gene expression regulators (e.g., RNA binding proteins, miRNAs, chromatin), giving rise to gene expression patterns that are highly complex.[10]

Some networks function in a modular fashion (such as a ribosome) but others, such as metabolic networks, do not have a modular organization. Because networks are hierarchical and because malfunctions usually happen at random and thus are more likely to occur in the abundant lower nodes, these scale-free networks tend to be robust against error. This contributes to systems (e.g., cells, tissues) that are robust against random perturbations and accounts for why a single malfunctioning gene has a low probability of impacting the system. Tumor progression involves interactions in multiple systems, making it unlikely that a single mutation will have sufficient pleiotropic powers to be solely responsible. Network structure provides a source of systemic robustness that decreases the likelihood that one renegade cell will crash the entire system.

The emergent properties resulting from interactions between networks allow an adaptability

of function that enables them to respond to the needs and demands of the surrounding environment. However, this interactivity of structure and function creates difficulties when it comes to confining pharmaceuticals to a single signaling pathway. Inevitably more than one target is affected and this can have both desired and undesired effects. Even with ideal targeting, some systems will respond as desired, while others will respond to the intervention by restoring the status quo, which in the case of an existing tumor, could be the perpetuation of a neoplastic microenvironment.

Cancer as an emergent property

The fact that emergent properties cannot be understood with reductionist methods has consequences for the study of complex diseases like cancer. If we focus on individual components of the system, we may get a very accurate assessment of how that particular part (gene, signaling pathway) functions under certain experimental conditions but still misinterpret its function with respect to the disease process. The analogy would be that of a blind man describing an elephant's tail in great detail and saying that 'elephants resemble snakes, because they are skinny and flexible'. The description is accurate but not representative of the elephant as a whole. In cancer research, it is critical to know how the part relates to and functions in the context of the whole; i.e., how a particular signaling pathway or gene functions in the context of a healthy or diseased intact system, and when in the process of initiation and progression it becomes important. Because complex diseases always have a time course, we need to know how its role evolves as the disease progresses.

If cancer is an emergent disease stemming from interactions between multiple physiological systems that also interact with environmental factors, then we need to complement reductionist methods with systems approaches that incorporate the micro- and macro-environmental context. Therefore it is critical to establish whether malignant tumors originate as localized phenomena (e.g., in the breast, lung, or prostate) driven by specific signaling pathways, or whether they are emergent manifestations resulting from the aberrant behavior of multiple interacting systems. Although cancer is currently staged by tumor size and development, it has been demonstrated that genome-wide methylation changes precede cancer and confer risk for cancer.[11] This would imply that 'stage 1' happens before tumorigenesis. These genome-wide epigenetic changes also imply systemic dysfunction. Two examples are the down regulation of mRNAs in the airways of healthy smokers that are consistent with mRNA expression changes in cancer[12] and persistent altered expression of a subset of miR-regulated genes in former smokers that is being attributed to their risk of developing lung cancer after they have quit.[9] The implication for treatment is that if we could identify a pattern of biomarkers that indicate dysfunction that is associated with increased risk for tumor formation, we might be able to prevent cancer development by restoring balance within/between these dysfunctional systems.

The implication of an emergent property view of cancer is that the system can influence a molecular or genetic target while a molecular change can also drive the system, meaning that causality is actually bi-directional. This is illustrated by a phenomenon well known to cancer researchers, namely that cancerous cells will often revert to normal when inserted into a healthy animal.[12,13] In fact, this is so frequent that animal models used in cancer research are genetically modified to assure reliable tumor growth. Experiments with these animals identify crucial pathways involved in the process but cannot convey information on how these pathways function in intact systems. The phenomenon of reversion suggests that it is not the properties of the cells, themselves, but the interaction between the cells and their surrounding microenvironment that determines malignancy. It illustrates an example of 'causality' that arises at a higher level of complexity (tissue) and changes the behavior at lower levels of complexity (e.g., the cell). The bidirectional causality that is a characteristic of emergent properties in complex systems is not consistent with most models of cancer etiology. Cancer models tend to be unidirectional, assuming that causality starts at the genetic or molecular level and progresses through ever greater complexity to the cancer phenotype. This difference in assumptions concerning causality is critical for modeling experiments relevant to treatment.

Attractors in complex systems

In complex systems, the phenotype is unpredictable because even minute and unmeasurable (potentially in

a quantum sense) variations in the initial conditions can cause a change in the outcome. Since it is never possible to know all of the initial conditions, the outcome is unpredictable even in completely deterministic systems. Thus, the key feature of nonlinear systems is that the response to a perturbation comprising multiple components or strengths cannot be predicted from a series of results of small, isolated perturbations. This is actually well-known in biology through genetic epistasis, however the implications have not been fully integrated into experimental protocols in cancer research or treatment. When we examine a specific signaling pathway in a genetically modified animal we are eliminating some of the interactions that occur naturally in intact animals and reducing the complexity of the processes we are studying. Because our model is limited in interactions, especially nonlinear systems interactions, assumptions that the results can be generalized to intact systems are premature at best.

Complex systems tend toward dynamic equilibrium, i.e., a state that is stable to perturbations due to attractors of the system.[1] An attractor is a concept in studies of dynamic systems that captures the fact that these systems, while constantly changing, tend to return to the same state repeatedly over time. Mathematically, the attractor is captured as a point in phase space, a convenient transformed view of the data. The best known attractor in biology is the cell cycle, in which growing cells repeat a path through states G1-S-G2-M, with minor variation in timing. This path appears in phase space as a slightly varying course around a point, the cell cycle attractor.

The attractor defining a healthy organism reflects an ability to respond and adapt to a changing environment. As an example, random mutations that occur in otherwise healthy organisms normally lead to DNA repair mechanism responses and/or apoptosis/anoikis, which destroy aberrant cells and maintain the system state. However, if the system escapes (is perturbed away) from its attractor, it is liable to take on new states defined by a new attractor, which may be equally robust against change. In the case of neoplasia, the cancer cell and microenvironment together move the system to a new attractor, promoting tumor growth rather than resisting it.

Organism as complex system

In sum, we expect the behaviors of a complex organism to evolve to a stable state described mathematically by an attractor. The stability of a healthy state has been driven by evolution to become robust to minor insults, such as infection, allergic response, minor wounds, etc, with specific exceptions where significant insults to the system, such as appearance of recessive genetic disease or undue burden from environmental exposure, perturb the state to a point where it is no longer stable. However, the state's stability is not inherent in the individual molecules or cells, but is an emergent property driven by the development of a system suitable for propagation. It is described as an attractor because the system (i.e., organism) returns to it after many minor insults that attempt to drive it to another state.

However, dynamic stability can be disrupted by environmental or biological insult as well as genetic disease. Once the system is driven to leave its current state of equilibrium, perhaps by weak but synergistic perturbations, the dynamics of complexity allow for a myriad of possible new trajectories to emerge. This will lead naturally to some diseases showing wide variation, in a version of Anna Karenina's principle: 'normal individuals are all alike but every diseased individual shows deregulation in its own way'. However, attractors will still exist, now in the form of dysfunctional states or phenotypes. What is important, and will be explained below in the section on systemic vulnerability, is that various trajectories can lead different diseased organisms to reach similar states. In advanced forms of cancer this state is often characterized by genomic instability. Unfortunately, once this new attractor is reached, it can be as resistant to change as the original, healthy state attractor, circumventing targeted attempts to inhibit growth by adapting and changing to override them. Treatment will fail if a full understanding of possible trajectories in the form of a systemic, mathematical model of the disease state is not attained. Such a model would allow simulations to be undertaken to explore a large number of potential interventions that could drive the individual back to the healthy attractor state.

Systems in an Organism/Vulnerability to Cancer

Only a minority of cancers are caused by germline mutations.[14] The majority (ca. 90%) are caused by somatic mutations and environmental factors, many of which are linked to inflammation and the

tumor microenvironment. Inflammatory responses appear to play a decisive role at every stage of tumor initiation and progression even though not all inflammatory disease increases risk for cancer.[14] Even dominant oncogenes will not induce cancer in adult animals unless accompanied by injury and subsequent tissue regeneration.

Inflammation and the tumor microenvironment

The transition from a healthy attractor to a carcinogenic one characterized by genetic instability and the hallmarks of cancer is influenced by a number of factors. Cancerous cells revert to normal in a healthy animal because healthy tissue will not support abnormal cell proliferation. This is an important issue not addressed by cell centric models.[15] In order for tumor cells to thrive they need an abnormal microenvironment, one that is characterized by altered extracellular matrix and non-transformed cells such as fibroblasts, myofibroblasts, leukocytes, myoepithelial, and endothelial cells.[16] The naturally occurring factors in the environmental substrate that normally inhibit tumor growth must be overcome. To understand what occurs when the natural defenses fail and a mutation is allowed to proliferate, we must understand more about the systemic conditions and microenvironments that foster tumor growth.

Inflammation can precede cancer as well as result from tumor formation, but it is almost always present in a tumor supporting microenvironment, even in tumor types not shown to be causally related to inflammatory disease.[16] The type of inflammation that changes the microenvironment, making it susceptible to tumor development, can be initiated by many different factors. Examples are infection (e.g., *Helicobacter pylori* and gastric cancer), environmental assault (e.g., particulates from tobacco smoke), or deregulation of the immune system and autoimmunity (e.g., inflammatory bowel disease). These types of perturbations can elicit an immune reaction involving cytokines, chemokines, growth factors, prostaglandins, reactive oxygen species, and nitrogen species, factors that create vulnerability for tumor formation and foster tumor growth.[14]

Cancer immunosurveillance refers to the multitude of cells and molecules involved in the recognition and destruction of cancer cells.[17] For mutations to survive and proliferate, these systemic damage control processes must malfunction or be overcome. That is why dysfunction associated with single genes or signaling pathways should be addressed in the context of where and when in the process they occur. Although immune and inflammatory cells can be found in almost all solid tumors,[18] their role is highly complex and not yet completely understood. The immune system plays a role in fighting tumorigenesis by destroying pre-malignant as well as fully transformed cells.[18] However, what starts out as an anti-tumor response can be subverted into a pro-oncogenic process—a potential path to a new attractor.

Once this process is started, cancer itself can subvert the immune system to aid in oncogenesis. An example of an anti-tumor response that is subverted to one that is tumorigenic can be found in myeloid cells. These cells can give rise to macrophages producing IL-12, an anti-tumorigenic substance, but can also differentiate within the tumor microenvironment to 'M2' macrophages that produce immunosuppressive and pro-angiogenic molecules.[18] When this happens inflammation can result from (rather than be a pre-condition for) disturbance of epithelial anchorage by proliferating cells or mutations. Just as injury to a vessel wall in the heart can initiate a wound repair response that derails, resulting in atherosclerosis, changes in the tumor microenvironment attempting to repair aberrant cellular events, can end up doing the opposite, namely promoting tumor growth. The extent of the complexity involved in the inflammatory contribution to carcinogenesis is further described by Gatenby and Gillies,[15] who note that carcinogenesis requires the surmounting of six distinct microenvironmental proliferation barriers for tumors to develop. A central role in the process is played by a form of apoptosis called anoikis, which is responsible for maintaining the correct cell number[19] and a healthy microenvironment by initiating apoptosis in epithelial cells that stray too far from their normal anchorage point in the extracellular matrix. The increase in diffusion distance created by cell migration from the basement membrane decreases the growth factors secreted by the membrane and initiates anoikis. Circumventing anoikis through aberrant signaling responses or genetic mutations[20] plays an important role in tumorogenesis.[21] If upregulation of glycolysis and resistance to acid-mediated toxicity do not

counteract the hypoxia caused by substrate diffusion distance, the process will be stopped and cancer will not develop. However, if the microenvironment adapts in a manner that supports tumor growth, microinvasion through the basement membrane will occur. To succeed, this must be accompanied by an upregulation of angiogenesis and other mechanisms such as fibroblast production of growth promoters and proteolytic enzymes.[15] What is interesting about this model is that it takes into account the diversity of mechanisms at each stage that can result in essentially the same outcome (phenotype), namely continued tumor growth, which forms a new attractor locking the system into a cancerous state and complicating effective treatment. According to Gatenby and Gillies,[15] "The environment selects for phenotypes, not genotypes, and multiple different mutations or epigenetic changes may produce similar phenotypes".

DNA repair mechanisms

This becomes even clearer when we examine DNA repair mechanisms and epigenetic mechanisms. All cells in the body except brain cells wear out and require periodic replacement. Given the high probability of replication error based on sheer numbers of replications and environmental insult, survival necessitates the ability to correct multiple types of DNA damage, such as breaks in the backbone of the double helix resulting in cross-links and other structural damage, errors in base replication, base pair deletions, etc. We have already discussed the form of apoptosis (anoikis) that stops tumor development by destroying proliferating cells not anchored to the membrane. There are also other types of apoptosis or programmed cell death, which are controlled or initiated by a myriad of complex processes such as stress, cytokines, glucocorticoids, growth factors, radiation, nutrient factors, and hypoxia, to name just a few. In addition, there are multiple other mechanisms responsible for DNA repair that can prevent the propagation of mutated cells. There is the recognition, removal and correction of DNA damage through base excision repairs, nucleotide excision repairs, double-stranded break repairs and repairs of interstrand cross-links.[22] There are also mechanisms for the activation of DNA damage checkpoints, whose function is to stop progression of the cell cycle so that the damage can be repaired before it is transmitted. To these can also be added transcriptional responses. Given these intricate defenses against DNA damage, it is clear that mutation alone is not sufficient to initiate cancer in a healthy organism.

Epigenetics and systemic vulnerability/ resistance to carcinogenesis

Although DNA sequence is hereditary and does not change throughout life, gene expression fluctuates dynamically based on cues from the external and internal environments. In fact, epigenetically driven changes in gene expression constitute a major part of the body's ability to develop diverse cell types and to respond to daily demands. For this reason, epigenetics has become an important area of cancer research. Its clinical importance lies in the fact that changes in gene expression can often be reversed by changing the environmental cues (such as diet) that trigger them.

Accumulating research now suggests that both genetic and epigenetic changes are involved in the etiology of cancer.[11,23–27] Methylation changes, that suppress or downregulate gene expression, have been shown to precede tumor development, as well as to predict risk for cancer.[11] Reported changes involve genome-wide hypomethylation as well as gene-specific hypermethylation,[11] both of which are associated with cancer.[28] Hypomethylation can result in oncogenes that should be suppressed, becoming expressed (e.g., as in loss of imprinting).[29] Hypermethylation on the other hand, can silence tumor suppressor genes that defend against cancer.[30] In a comparison of normal colonic mucosa with that from colorectal cancer, most of the 33 loci investigated in normal tissue were methylated due to age but underwent more extensive methylation in cancer.[31] However, methylation of 7 loci was limited to neoplastic tissues and found to be clustered in a subset of cases characterized by microsatellite instability (MSI),[31] i.e., abnormally varying lengths of repeated DNA sequences resulting from faulty DNA repair. A review of the literature[31] shows that this type of methylation clustering appears in gastric cancer, ovarian cancer, esophageal cancer, liver cancer, pancreatic cancer, lymphocytic leukemia, and acute myelogenous leukemia. The rates of tumor-suppressor gene hypermethylation (inactivation) vary significantly across cancer types. Such changes have

the potential to be more adverse than nucleotide mutations because they can spread in cis-regulatory elements.[31,32] However, the process is dynamic in the sense that there are also mechanisms in the CpG islands that fight the spread of methylation and defend against changes that would promote tumorigenesis. Similar to avoiding apoptosis, the occurrence of dysfunctional hypermethylation requires bypassing normal defense mechanisms. Thus, epigenetic mechanisms play a key role in emergent properties because of their influence on gene networks.

As an example, anoikis can be epigenetically inhibited,[19] and genes serving as key integration points in networks are especially susceptible. The p53 tumor suppressor gene can be influenced by many different signals[33] and the cellular response can vary from DNA repair, to cell metabolism, autophagy, apoptosis, and cell cycle arrest.[34] If it is methylated, it no longer functions as a tumor suppressor, becoming equivalent to a deleterious mutation.

Epigenetics and lifestyle factors

The importance of epigenetics lies in the fact that changes in gene expression are influenced by reversible lifestyle factors. Diets high in fried foods and red meat, cigarette smoking, sun exposure, obesity, physical inactivity, and environmental pollutants have all been found to increase cancer risk,[35] and they all drive changes in gene expression. This is illustrated by the known association between sun exposure and skin cancer. Animal research has demonstrated a distinct hypermethylation pattern associated with UVB radiation in epidermal skin and UVB-induced skin tumors that causes increased expression of DNMT 2 (TRDMT1), DNMT3A, and DNMT3B.[36] These changers in methyltransferases are associated with histone modifications and silencing of tumor suppressor genes $p16^{INK4a}$ (CDKN2A) and RASSF1.[36] However, certain dietary components have been demonstrated to protect the skin against UV radiation. Epicatechins found in green tea and proanthocyanidins from grape seeds have been demonstrated to block hypermethylation of $P16^{INK4a}$, p21, and cip1 (CDKN1A) tumor suppressor genes,[37] thus reducing risk.

The role of dietary nutrients on methylation cannot be overstated. Not only are dietary patterns independent predictors of cancer risk, they also interact with other exposures, such as viruses, to increase or decrease risk for cancer by changing DNA methylation. It has been demonstrated that an unhealthy dietary pattern can increase risk for cervical interepithelial neoplasia by 3.5 times.[38] Dietary patterns were defined by factor analysis. An unhealthy diet consisted of intake high in sugar beverages, pasta and starchy foods, margarine, butter, refined grains, sweets, and snacks with high dairy fat. That same study reported that women who were infected with the human papillomavirus, which increases risk for cervical neoplasia, but who had very healthy dietary patterns (defined as seafood, beans, lentils, tofu and other meat substitutes, whole grains, fresh fruits, canned fruits, vegetables, peanut butter, low fat dairy, chicken and turkey, cereals, yogurt, and phytochemical rich foods) had a reduced risk of clinical manifestation, i.e., were 3.3 times more likely to have methylation (repression) of a biomarker that is predictive of increased risk for this type of cancer, than women with the unhealthiest diet pattern. These clinically relevant interactions indicate the importance of approaching treatment and prevention from a systemic perspective.

Allostatic load and breakdown of dynamic equilibrium

The term allostasis has been coined to describe the dynamic ability of the body to maintain stability despite changing conditions[39] and is integrally linked to the concept of an attractor. The body has many systems that are designed to meet and satisfy the daily physiological challenges, which include a plethora of factors from viruses and bacteria, to mutations, obesity, aging, and stress. As long as demands made on the body do not overwhelm the systems' resources and ability to respond, a state of dynamic equilibrium and healthy functioning is maintained. This is one reason that moderate physical activity, healthy diets, refraining from smoking, and moderate alcohol consumption are associated with lower risk for chronic diseases than lifestyles that involve habits of overconsumption and poor nutrition. Under normal conditions, the robust flexibility built into an organism creates methods to compensate for an overloaded system by supplying back-up systems that can temporarily compensate. The flexibility of the p53 tumor suppressor gene is a good illustration of this.

However, when challenges become too great or unrelenting (e.g., smoking continues for many years), the organism becomes overwhelmed. Activity in multiple systems then increases and is re-directed in an attempt to compensate. If the problem is not resolved, eventually the feedback within and between systems begins to break down. Restorative mechanisms that normally keep systems functioning properly are overused and catabolic processes begin to predominate. Allostatic load is the burden on tissues and organs that accumulates from chronic over-activity. Eventually it can perturb systems away from their healthy attractors toward deregulation characterized by a breakdown of feedback mechanisms required to maintain balance.[40,41] The overuse of compensation/defense mechanisms can cause a transition from a healthy to an unhealthy attractor, subverting the body's defenses towards maintenance of an unhealthy state. This can be illustrated by the types of transitions involved in inflammation and metabolic syndrome. The fact that genome-wide hypomethylation as well as hypermethylation precede cancer indicates that multiple systems are affected prior to tumor manifestation. An ability of the tumor microenvironment to adopt and maintain a dysfunctional state may be the primary characteristic of cancer.

To summarize the process, the body maintains many defenses against tumorigenesis. Malfunction in multiple interacting systems is required for tumor growth to occur, and this can be illustrated by the types of cancer that are known to be associated with environmental toxins or viruses. Although smoking is a well-documented predictor of lung cancer,[42] only 10%–15% of smokers develop it and most smoke for decades before lung cancer manifests.[43,44] Similarly, the increased risk of cervical cancer in women who test positive for the HPV virus is well known. However, although approximately 26.8% of American women test positive for HPV,[45] the yearly incidence rates of cervical cancer are about 7.5%[46] and a great many women who test positive for this virus can carry it for many years before developing cancer. These examples suggest that host susceptibility plays an important role in determining risk for cancer. A strong, robust system will be resistant, while a less robust (e.g., aging) system will be more susceptible. These data illustrate that it is the interaction between the cell and other systems, not the properties of the cell itself, that determine whether malignancy will develop, and clarifies why a cancer cell inserted into a healthy animal can revert to normal. In such cases, the system level characteristics dominate over those at the cellular level.

Implications for Treatment

Targeted treatment

The advent of imatinib mesylate (IM) for the treatment of CML was a watershed moment in cancer therapeutics, converting a certain death to a prolonged and potentially normal life.[47] IM functions by blocking activity of the tyrosine kinase encoded by the BCR-ABL transgene on the Philadelphia chromosome,[48] effectively blocking the constitutively active growth signal that drives CML etiology. The miracle of this treatment led to an explosion of research into tyrosine kinase inhibitors (TKIs) targeting other deregulated and mutated kinases functioning in growth and anti-death pathways,[49] which are critical components of cancer.[50] In addition, a great deal of effort has been focused on development of therapeutic antibodies to receptor tyrosine kinases (RTKs) that often trigger growth signal cascades.[51] However, as noted above, the results have not been nearly as dramatic as in CML, with improved survival generally measured in tens of months.

TKIs are typically SMTs, and the targeting of such molecules is generally not precise. Off-target effects have been identified,[52] and in many cases carefully designed SMTs turn out to be marketed as multi-kinase inhibitors once more of their interactions are deduced.[53] The targeting of multiple kinases can be beneficial however it raises a question of the mode of action of the therapeutics and creates difficulties for understanding tumor response.

Recently, it has been shown that successful targeting of a kinase can also unintentionally increase off-target signaling by shunting upstream signals into parallel pathways.[54] We have also demonstrated off-target effects through activation of distinct rescue pathways in both cell lines and patient tumors.[55] Overall, the result of a dramatic research effort to identify kinase targets, develop targeted SMTs, refine pharmacodynamics and pharmacokinetic aspects of the SMTs, and move SMTs to clinical trials has not had the desired dramatic impact on cancer survival.

Both our limited understanding of the molecular state of the system and a lack of adequate nonlinear models of cancer systems and their interactions with the host have led to an inability to predict pathway changes in response to therapy, highlighting a need for a systems approach.

As most of the research on targeted therapies has been done in modified (e.g., knock-out) animal models with compromised immune and other systems because of the difficulty of growing tumors in otherwise healthy animals, the early removal of the system during drug development may be contributing to the limited success of SMTs. For most cancers, there is not a single hit, such as BCR-ABL, so the cancer system has evolved to a more complex state with more developed interactions with other systems in the organism. Our models must address this complexity in order to design methods to escape the cancer attractor.

Network pharmacology

One approach that has begun to address this issue is the emergence of network pharmacology. This branch of pharmacology utilizes research from multi-pathway interactions (e.g., genomic, biochemical, etc.) at multiple scales (molecular, cellular, tissue, organ) to design drugs with inter-related targets for cancer therapy.[56,57] Arriving at these solutions involves multiple quantitative measures, which include connectivity (how many links a node has with other nodes), degree distribution (a probability obtained by adding the number of nodes and links and dividing by the number of nodes), an estimate of the degree exponent or importance of a node in scale free networks, the shortest path and the mean path, and a clustering coefficient.[58] This is in essence, a systems pharmacology approach involving multiple levels of interaction from genomics through proteomics and metabolomics to the organ level. It is an enormously complex process because it involves the identification of targets that bind or metabolize structural variants of a drug differently. The FDA Adverse Events Reporting System Database and other databases such as DrugBank are currently options for identifying some of these targets.[57] Although the field is still new, it holds tremendous potential for future treatment options. However, if network pharmacology is to succeed, the potential for multiple agent treatments to disrupt essential normal processes in off-target cells types, such as innate immune cells, must be monitored and managed, as these effects can lead to comorbidities and death.[59]

Quantitative model of a system that captures adequate complexity for predictability

The creation of a model that can truly simulate the behavior of any living system is beyond our present capacity for a number of reasons. As such, we must instead focus on creating models that balance our ability to determine adequate relationships, scales, and parameters with adequate coverage of possible system states to make meaningful predictions in the nonlinear regime. Most models in cancer have focused on high-level behaviors[60,61] or on single pathways.[62] Traditional biochemical models utilize stochastic versions of the chemical master equation[63] to track chemical reactions and species, which require substantial knowledge of in vivo rate and binding constants, which are generally still poorly elucidated. A compromise is to produce a hybrid model that combines models with limited parameterization (e.g., graphical models) for cell signaling with traditional models for expression to try to capture enough complexity to predict complex, multicellular behavior.[64]

From this hybrid approach, the outline of a quantitative model can be predicted. The model cannot rely on detailed biochemical knowledge in cancer or other cells, since these measurements are unlikely to be made soon. The model must therefore abstract away much of the behavior, where possible in the form of a graphical model when it is adequate to capture behavior (i.e., a stochastic graphical binary model for signaling where each protein is either "on" or "off"). This simplification cannot be made in the realm of gene expression, as protein concentrations generated by transcriptional reprogramming and translational control are inherently non-binary. System interactions can be modeled in multiple ways, from endocrine signaling through diffusion and transport through vascular systems to juxtacrine and autocrine signaling based on cell location. Overall, the model must therefore capture both spatial and temporal relationships at scales from the organism through the tissue to the molecular. Creating such a

model will be a major undertaking requiring a team science approach. However without such a model our progress in treating advanced cancer is likely to remain limited.

Presently, ordinary differential equation (ODE) models can be used to study the growth of cancer in organisms[65] or limited sets of signaling pathways in cells,[66] generally by creating de novo models and comparing predictions to measurements. Evolutionary models allow exploration of the potential of individual clonal populations to grow, given limited resources and competition.[61] Graphical models, such as Bayesian Network (BN) models, permit identification of potential pathways and proteins that drive transcriptional reprogramming in a data-driven approach.[67] In the future, predictive models useful for refining treatment will build on the aforementioned modeling approaches but also include pharmacodynamic and pharmacokinetic models[68] within a dynamic systems model. The final form of such a model can be predicted by the similar needs in weather forecasting, where complex dynamical models are updated routinely through Kalman filtering, which updates a model's state parameters through observations.[69] In the case of cancer therapy, these updates would be routine clinical and molecular measurements made during treatment and subsequent monitoring.

Conclusions

In conclusion, the current treatment paradigm of trying to discover the cause of a phenotype by identifying single key genes or pathways that explain it has not been very successful. The fact that the field of oncology has been cited as having one of the poorest investigational drug records in clinical development[70] is cause for us to pause and examine whether the paradigm we are using might need revision. The assumption that destruction of cancerous cells or even tumors should be the method of choice may be misguided.[1,70] This is the frame of reference we use to fight other organisms such as bacteria and viruses that invade our bodies and cause disease. However, tumors are part of our own bodies and pharmaceuticals that destroy one part have a high probability of negative side effects on others.

Accumulating data indicates that making headway in cancer treatment will require progress in multiple areas: (1) better profiling of systemic pre-cancerous biomarkers associated with elevated risk so that preventive lifestyle modifications can be instituted early to revert high-risk epigenetic changes before tumors develop; (2) a treatment emphasis in early stage tumors that would target the restoration of systemic balance, not through destruction of pathways but through strengthening the body's innate defense mechanisms and perturbing the system back to a healthy attractor; and (3) establishing better quantitative models of systems to capture adequate complexity for predictability at all stages of tumor progression.

Trans-disciplinary collaboration is the only solution for developing a systems biology approach that incorporates the body's dynamic, interacting response systems in the healing process. The recent work of Gillies, Verduzco and Gatenby,[71] which suggests that evolutionary dynamics of cancerous cells drive the lack of success of targeted therapies, is related to but different from our view that dynamic systems within the host must be disrupted for cancer to develop and grow. Interestingly, both our perspective and that of Gillies, Verduzco and Gatenby require a new approach based on collaborative groups that address biological dynamics with mathematical models.

Competing Interests

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Wrote the first draft: SSK. Agree with manuscript results and conclusions: SSK, MFO. Jointly developed structure: SSK, MFO. Made critical revisions and approved final version SSK, MFO. All authors reviewed and approved of the final manuscript.

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation

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