Opinions

Gene, Life, and Disease - A Node Theory

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Abstract: The 21st century embraces a combination of the Genomic and Information Ages, characterized by enormous genetic data deposition and use of numerous powerful analytical tools. With more and more accumulated data, the concept of genes needs to be redefined and research methods further rationalized based on the properties of life: economy, optimization and survival. Disease should be considered the condition where homeostasis is disrupted. Biological equilibrium is maintained by a network of numerous functional nodes: molecules located in various pathways that are classified into decision nodes and alternative nodes, which shift their roles in response to a changing environment. Organized both spatially and temporally, the nodal network carries out its functions for the optimal outcome of the cell. Although central nodes often play vital roles for cell functions, connector nodes in the "structural hole" are more effective targets for treatment. Resources should be prioritized to delineate the roles of functional nodes and identify critical nodes for optimal therapy.

Introduction

It is said that in ancient India a prince sat under the Bodhi tree and realized the secrets of life. Commonly known as the Buddha, among his teachings was the story of four blind men who were brought before their king and asked to describe an elephant [1]. Upon touching the different body parts of the elephant, the four blind men drew different conclusions of the object they felt. None were correct, as each man could only deduce based on their incomplete knowledge. This story reminds us of the many scientific breakthroughs during the last several decades. With the sequencing of the human genome and development of DNA microarray technology and various "omics", innumerous genes, RNAs and proteins are being studied every day in hundreds of organisms, generating an enormous amount of data whose entirety can barely be analyzed by the most massive of supercomputers. With a vast size of information readily available from advanced technology, are we able to embrace this opportunity to uncover the secrets of gene, life, and diseases?

Molecular biology has endowed humans with the ability to dissect life at the molecular level. Application of isotopes and fluorescent proteins enables the tracing of biological processes in the cell at any given moment. However, most contemporary biological studies capture mere snapshots of life. Because of this, data is largely pieces and frozen. Many researchers recently attempted to track cell activities in real time [2, 3] but limited to the use of assays, only one or two factors are examined at one time. When scientists try to combine individual information to form a coherent summary of their findings,

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the information itself is often fragmented with so many gaps that prevent a complete picture from being built. To counter this, tracing technology has been applied to the clinical diagnosis of cancer (such as PET scan), it is currently only used for small and simple molecules, such as glucose. Since genes and proteins are comparable in complexity to the stars in the universe, it is impossible to trace all the biological reactions by presently available technologies.

To study the effects of a single gene, we often try to keep the other genes constant. This is the so-called "one gene at a time" [4]. Based on this traditional logic [5], researchers invented transgenic, knock out, and genetic engineering technologies to temporarily or conditionally break the equilibrium of biological systems, in order to define the functions of affected genes. However, although these approaches do affect the cells as a whole, they do not necessarily reveal the actual functions of the genes. This is illustrated in Figure 1, which is the "input A/output B". Because a black box, representing the lack of constant monitoring of the process, exists between the cause A and result B, the same gene could lead to different results in different organisms, or even in the same organism but in different laboratories [6]. This issue is apparent with the *MYC* gene, which has been shown by various studies [7–22] to cause both tumor proliferation and apoptosis - two seemingly opposite effects. These examples reflect the limitations of the current scientific method:

1. Mere snapshots of dynamic physiological processes;

2. "One gene at a time" with the assumption that everything else remains the same;

3. Change one gene and assume the outcome is the consequence of that gene.

These restrictions are exemplified by the failure of the Merck HIV vaccine [23] as well as the Eli Lilly γ-secretase Alzheimer trial [24].

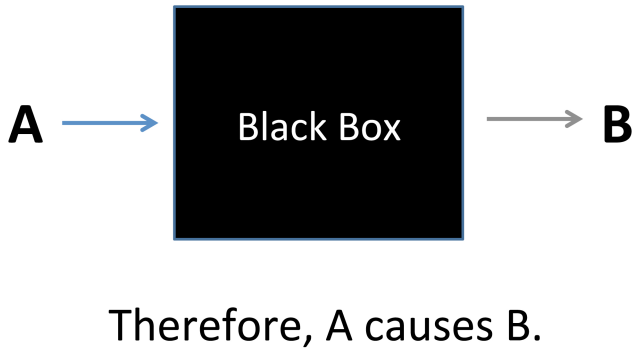

Figure 1: A simplified John Stuart Mill's inductive methods. However, the process in the black box may significantly change the outcome B if A is not well standardized, which could account for the irreproducible research results.

Genomic Age

DNA

Watson and Crick's DNA double helix [25] brought humans into the Genomic Age. Every organism possesses genes, which determine its biological functions. Gerstein *et al*. defined a gene as "a union of genomic sequences encoding a coherent set of potentially overlapping functional products" [26]. An organism's complete set of genes make up its genome. The entire human genome has been sequenced [27] and contains 50,000 - 100,000 genes [28]. About 20,000 encode proteins [29], making up only 1-2% of the total genome [30], while the rest of the genome is mostly composed of regulatory or coding sequences for peptides with yet unknown functions [31]. While the debate over the "junk" gene rages on, it is still difficult to believe that the number of human genes is comparable to the number of chicken genes, and much less than that of a grape [32]. Does this mean that the number of genes is irrelevant to an organism's complexity? It does not if a gene is no longer equivalent to a segment of DNA, but rather a nucleotide sequence that determines the function of its end product - RNA or

protein [30].

From Gregor Mendel [33] to Oswald Avery [34], generations of scientists have tried to define the function of a gene. The creation of transgenic animals and gene targeting techniques opened the door to modern advancement in studying the functions of individual genes [35, 36]. In the transgenic animal model, a particular gene is introduced into the embryo to produce a line of offspring that constitutively or inducibly expresses that gene. The phenotype expressed by the resultant animal is presumably then the function of the gene. In the gene targeting animal model, the function of a particular gene is disrupted by "knock-out" to show the phenotypic changes in the resultant animal. Both methods artificially interfere with the natural genetic homeostasis, an important property of life, but are so far the best approaches to elucidate the function of an individual gene.

A segment of nucleotide (or gene), when acquiring a point mutation that changes the function of the encoded protein, is considered a different gene; this is called polymorphism. In this sense, the complexity of life is determined not by the number of open reading frames, but by the functional diversity of its genome. A genome's functional diversity is defined as the genetic reserve of the organism: with the same segment of DNA, a point mutation can change the function of the encoded protein; a frame shift by a single nucleotide deletion or insertion could lead to the production of a completely different protein, which could also result from the alternative splicing of mRNA and alternative initiation of translation in protein synthesis. This definition emphasizes that the gene is a segment of nucleotide that encodes the smallest functional unit of life.

RNA

Between genes and their functional end product proteins, RNA acts as an intermediate as the product of transcription and replication, and the blueprint of translation. A gene can be an RNA sequence, such as in RNA viruses. When injecting a small interference RNA molecule into the round worm, Andrew Fire and Craig Mello opened a new frontier of molecular biology and molecular medicine [37, 38]. Subsequently, microRNAs were identified as regulators of genes [39] and possible causes of diseases [40]. Therefore, RNAs are genes and gene regulators.

Protein

Protein is at the downstream far end of the *Central Dogma*. With the exception of prions, proteins can neither replicate nor be copied backward into DNAs or RNAs. Their production or degradation, modification or de-modification plays an important role in determining the fate of cells. Since most of the activators and inhibitors of gene expression are proteins, they perform the most important regulatory functions in life, and are subjected to most of the uncertainties and variations. Thus, *proteins are the ultimate targets for modifying gene functions*.

Big Data

Advancements in computer science marked the transition to the Information Age. Using automation, we not only sequenced the whole human genome [41, 42], but also plan to transform the future through nanobiology [43] and personalized medicine [44]. However, what should we do with all of the identified genes and proteins? Some proteins have already been the targets of cancer therapy. For example, the success of imatinib [45] set a molecular therapeutics precedent for designing drugs based on molecular discoveries. Innumerous drugs are being tested every day to target various molecules that cause diseases such as cancer, diabetes, Alzheimer's disease, etc., but only a limited number of these drugs has proved to be clinically effective [46]. How should we rationalize the process of discovery? Fortunately, this answer lies in life itself.

Life

What Is Life?

We are privileged and humbled that the earth is the only planet we know of that sustains life, but how do we define life? Besides the characteristics described by Nealson and Conrad [47], life has the following properties:

1) Economy - Life is efficient at conserving energy. Under unfavorable environmental conditions or when food becomes scarce and continued activity and intensive metabolism would result in extreme exhaustion, organisms will enter dormancy, a state of low metabolic activity. After a big meal, the liver will send all the lipid-binding proteins out to collect the fat and store them in the liver and adipose tissues. Free sugars will be polymerized and stored in the form of glycogen. When energy is required for strenuous activity, glycogen will break down into glucose to provide energy to the muscles. This property is spontaneous and innate in life.

2) Optimization - It is easy to marvel at how delicate and complicated life is, seemingly a result of pure chance. From the simplest virus to the highly advanced human, everything appears to be so well designed. However, life developed itself during the long period of evolution [48], the process by which life obtained the property of optimization. Only the most suitable organisms survived under the pressure of natural selection; suboptimal life either adapted to their environment or went extinct. The fact that only 1% of our genes encode functional proteins [30] led many scientists to believe that the rest of the genes are "junk" [49]. However, with more and more evidence showing that regulatory RNAs play important roles in life, it is almost universally agreed that every bit of our DNA is required for life [31]. This apparent "junk" DNA in fact provides the genetic reserve for life to evolve to its optimization.

3) Survival - All organisms constantly face the challenge of survival. From birth to death, an organism always tries to adjust itself to fit its ever-changing environment, repeatedly weighing the harm and benefit of various situations, making numerous decisions, and sometimes modifying those decisions. For example, when the E→V mutation caused the formation of a sickle shaped red blood cell [50, 51], this defective feature remained because of its advantage against plasmodium that allowed for survival in the harsh malaria epidemic environment in Africa and South Asia. When confronting the options of survival and death, life always attempts to live, even when death is imminent such as in an aged or diseased organism. However, death is only another step in the cycle of life, with an organism's offspring continuing to survive. Complex organisms tend to live longer while simpler life forms survive through enhanced reproduction. Reproduction is another, longer-lasting means of survival in species. Life is a masterpiece of teamwork, with members including all the molecules that constitute the integral body and occasional visitors that contribute to the haps and mishaps of life.

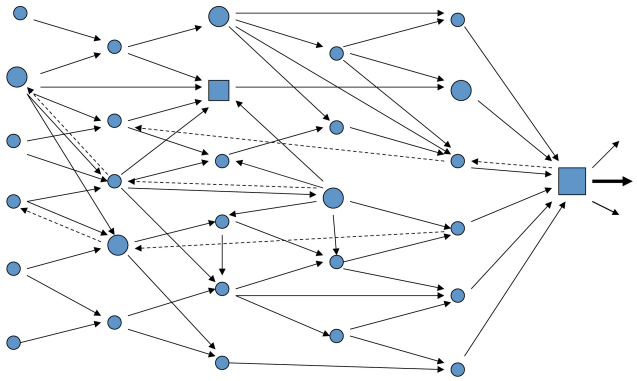

Figure 2: The signal transduction network in a single cell. The circles depict alternative nodes while the squares indicate the decision nodes. The arrows show signal flow directions while the broken arrows mark the reverse flow of signals or feedback signals.

Life Is A Decision

Life constantly makes decision about economy, optimization and survival. Decision making is often automatically controlled by programmed genes and gene products - the functional "nodes" [52]. Interactions between functional nodes form the functional network. Cooperation and communication between the nodes keep the organism in homeostasis. Broken biological equilibrium will lead to disease, and complete loss of equilibrium will eventually lead to death. Nodes use the most efficient language (binary language) for communication and decision making.

Binary Language

The earliest binary language can be traced back to the ancient Chinese classic Yijing (Book of Changes) [53], which uses Yin and Yang, sun and moon, sky and earth, bright and dark, presence and absence, have and have-not, to express the opposite extremes. The modern computer also uses binary language in the form of "1" and "0". Binary language is not only simple and qualitatively sound, but also easy for conversion and efficiency. Life also utilizes a system similar to binary language during evolution. Genes are turned "on" and "off" by activators and inhibitors; protein can be activated or inactivated with phosphorylation or dephosphorylation by numerous protein kinases or phosphatases; cells divide or fuse; organisms live or die. There is no third option.

Pathways Or Network

With innovative development of various assays, numerous pathways have been identified. The most well studied ones are signal transduction pathways that function as a cascade. More and more works show crosstalk between different pathways, placing doubt on the existence of pathways [Figure 2]. Most current biochemical research data was obtained *in vitro*; participating molecules were usually isolated from living cells, changing physiological functions

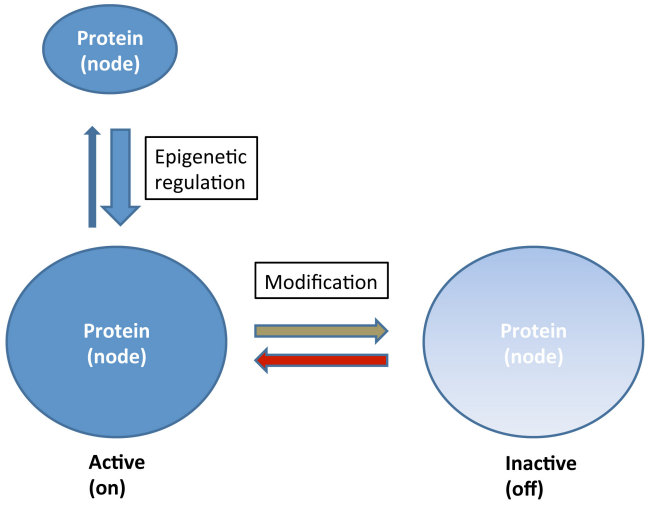

Figure 3: Regulation of nodal function is both qualitative and quantitative. Qualitative regulation is "all or none" and can be carried out at the genetic and protein levels. Quantitative regulation can be measured in scales and realized by point mutations, transcription activation or inhibition, modification of protein activities.

into chemical reactions. Thus, the collected data reflects only a snapshot of life, akin to the blind men feeling an elephant. One lab found the "tusk", the other felt the "trunk", and another grabbed the "tail"; we still have not seen the whole picture. One external stimulus may affect hundreds of different molecules in a cell; some changes are detectable whereas others remain unseen with current technology and cannot be factored in during analysis. These molecules interact with each other to form a biological network, which keeps life in homeostasis. When homeostasis is disturbed, life will malfunction and suffer from diseases.

Diseases

Why do we get sick? The ancient Greeks regarded diseases as curses that escaped from Pandora's Box [54], except for Hippocrates, who believed disease was not a punishment of gods, but instead an imbalance of man with the environment [55]. This concept

of disease was shared by the ancient Chinese, who perceived disease as a functional disharmony (or imbalance) in the human body itself or as a result of interactions between the body and the environment [56]. Disease is often construed as a disorder of structure and function in an organism, associated with specific signs and symptoms. The modern concept of disease, although more successful in practice, misses the essence of disease - an "imbalance of life". As a result, we sometimes treat one disease yet cause another. Indeed, disease represents a loss of homeostasis in life. Disease is also a natural force that selects new functions which may help life to survive in a new harsh environment. When the E→V point mutation first occurred in a hemoglobin molecule, a new functional protein that caused the sickle cells appeared. If it was not owing to the selecting pressure by malaria, this mutation might not have survived. Disease can also be regarded as a process of abandoning harmful mutations. Some lethal mutations lead to abortion, adolescent death or infertility, thus preventing its propagation in the offspring. By prolonging the life of individuals with genetic diseases modern medicine ironically helped to conserve some of those harmful mutations. Since disease is inherent in life, when an organism dies by disease, it is considered a death from natural causes.

Cancer is a very complicated disease that involves multiple molecular events, activation of various oncogenes and/or signal transduction pathways, which poses a great challenge to treatments. With a few exceptions, the mainstays of current therapy are still cytotoxic agents, which are usually toxic mutagens or carcinogens that kill the fast growing tumor cells and damage some normal cells at the same time. Thus, cure of the primary cancer could lead to a secondary malignancy [57]. Advancement of new technologies such as genome sequencing, microarray and proteomics brought up enormous amount of information on almost all the pathways that have been activated in cancer. Pharmaceutical companies have designed and synthesized hundreds of small molecules that target the components

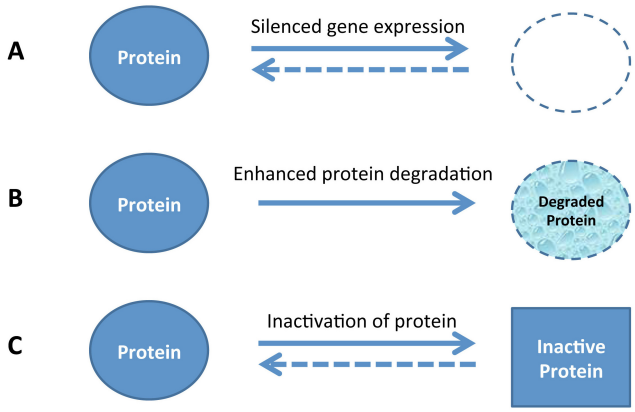

Figure 4: Structural "hole" - the shunt of signals. In normal cells, the signals flow from cluster A to cluster B to cluster C and *vice versa*. Due to the oncogene product (black filled circle) that creates a shortcut between cluster A and cluster C, the cancer cells acquire proliferating advantage over their normal counterparts. The signals in cancer cells will bypass the regulation by cluster B and thus targeting cluster B will be ineffective.

of the activated pathways. However, to target all those molecules is neither practical for physicians nor tolerable for patients. Based on our studies and those of others, the concept of the functional node is proposed to rationalize the targeted therapeutic strategies.

Functional Nodes

Definition

"Node" originated from the Latin nodus ("knot"). A simple "node" can imply many different meanings. A node in physics is a point along a standing wave where the wave has minimal amplitude [58], whereas in computer science a node is an interconnecting point of a network [59]. I have used node to indicate the point where different signal transduction pathways crosstalk [52]. Here, a "node" refers to a junction that relays signals from one functional unit to another in a living cell. Although nodes can be any molecules that form an integral part of a

functional network, here I focus on proteins. Every node is unique in carrying out its function(s), and is dynamic that changes with the natural evolution. Based on their predominant functions, they may be divided into decision node and alternative node, as we define a decision tree [60]. The interactions between all the nodes determine the final outcome of a cell, an organ and an organism.

Node Functions

Nodal functions can be regulated both qualitatively and quantitatively [Figure 3]. For example, a node can be activated by either increased production or newly acquired mutation(s), and inactivated by deletion, silenced gene expression, molecular modification (such as acetylation, methylation, and phosphorylation, etc.), and degradation [Figure 4].

The functional importance of a node depends on how often it is affected by a certain stimulus and its location in the molecular network, which can be depicted by several functional points (Table 1). Two types of functional nodes exist: the decision node and the alternative node. The decision node acts qualitatively and exerts its function by an "all or none" mechanism, whereas the alternative node functions in a measurable way. Based on the number of stimuli and the strength of each stimulus it receives, the alternative node can have dose dependent effects or even opposite effects. The net effect of an alternative node can only be measured under an ideal condition. If an ideal condition is not available, the effects of an alternative node must be tested repeatedly to obtain a reproducible result. When the probability of the tested results approaches 100%, an alternative node becomes a decision node.

Shifting Roles

Cells are dynamic and the node function can shift from alternative node to decision node under certain conditions; the roles of the nodes may change around clock with cell cycle progression. A drug that works today may not work anymore in a couple of years, or months. Therefore the nodes should be monitored constantly and the node targeting should be adjusted accordingly.

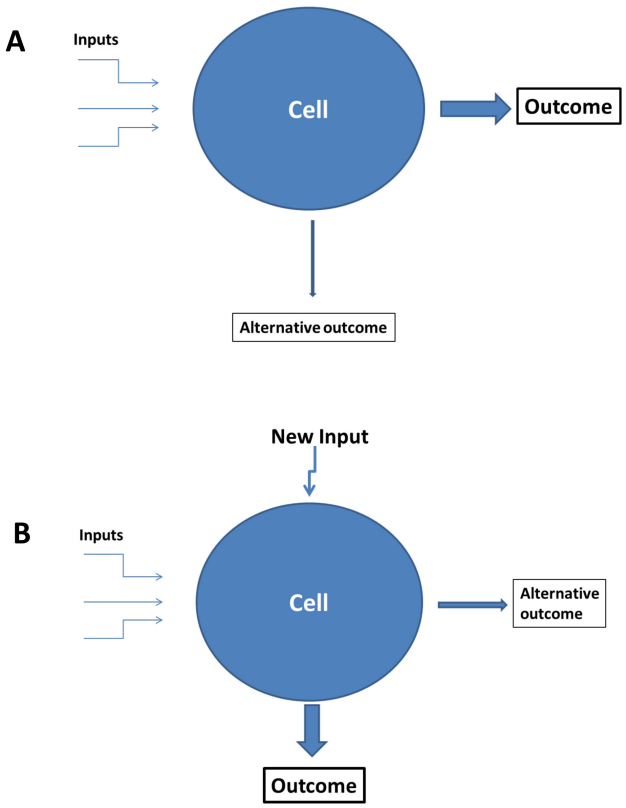

Figure 5: Change of the node functions. (A) Decision node dictates the cell outcome; (B) With certain stimulus, alternative nodes can take over the role of decision node and switch the cell to an alternative outcome.

External stimuli affect the cell function and the roles of various functional nodes. With a changing environment, the nodes may shift their roles from alternative nodes to decision nodes, or *vice versa* [Figure 5]. The environment could be temperature changes, osmotic changes, growth factors, and drugs. Study of the shifting patterns of the functional nodes will predict the responses of the cells, thus to regulate the cell functions or target lethal nodes to kill the cells. Changes in the environment will trigger the cells to activate a protective mechanism - feedback. Feedback is one of the most

important mechanisms in the living cells, often in response to neuroendocrine factors. When the cells are stressed, they often make extreme decisions for survival. Bacteria and tumor cells know how to activate their multi-drug resistance (MDR) genes to pump the toxic drugs out of the cells [61], just like sailors trying to save their sinking boat by bucketing out the sea waters from the cabin. Shutting down the feedback mechanism in the bacteria and tumor cells is critical for therapy.

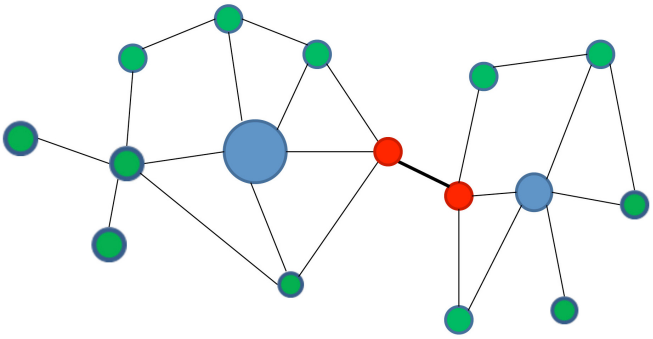

Figure 6: The connecting nodes - key players in the network. In a functional network, key players that connect two or more clusters of network play critical role in signal transduction and cell survival.

Organized Hierarchy

Although a clear pathway might only be transient, the nodes are organized in a hierarchical fashion. Compartmentalization by nucleus and other organelles separates the molecules into different functional groups. The functions of some nodes are well controlled by modification and degradation, whereas those of the others are protected by chaperones against the hostile environment. No example is better than the process of development and aging. At the moment of conception, life starts to organize its functional nodes; genes are expressed in a chronological order. If a node function is missed or skipped in this process, life may not be able to meet its milestones and will have developmental defects. The on and off of the genes is orchestrated so well which even outperforms the most talented conductors.

Optimization

Functional nodes form a dynamic network in which each node plays a role for the optimal outcome of the cell [Figure 2]. Node interactions can be unidirectional, bidirectional or in a reflex arc. The strength of interactions may also vary from minimal stimuli to a radical shock to the cell. All these interactions normally are coordinated towards optimization. However, when mutation occurs the mutated molecule may disrupt the optimal interaction between nodes and lead to chaos in the cell. Even when this does occur, the cell will first try to fix the problematic nodes and to recover from the chaos. One major player is p53, which is activated by DNA damage [62]. If the cell could not fix the chaos, it will choose either to die (apoptosis) or to tolerate the chaos (cancer).

Network

Every node is unique, but some nodes have more strength than the others. When signals arrive from upstream, not every node will respond the same; only when a threshold for a node is reached the

Table 1: Definition of functional points

Functional Points	Definition
Tilting point	the molecule at which two major opposite effects meet with similar strength
Facilitating point	the molecule at which two major similar effects meet with additive or synergistic effects
Vital point	the molecule at which all the critical effects can be activated
Lethal point	the molecule at which all the critical effects can be inhibited

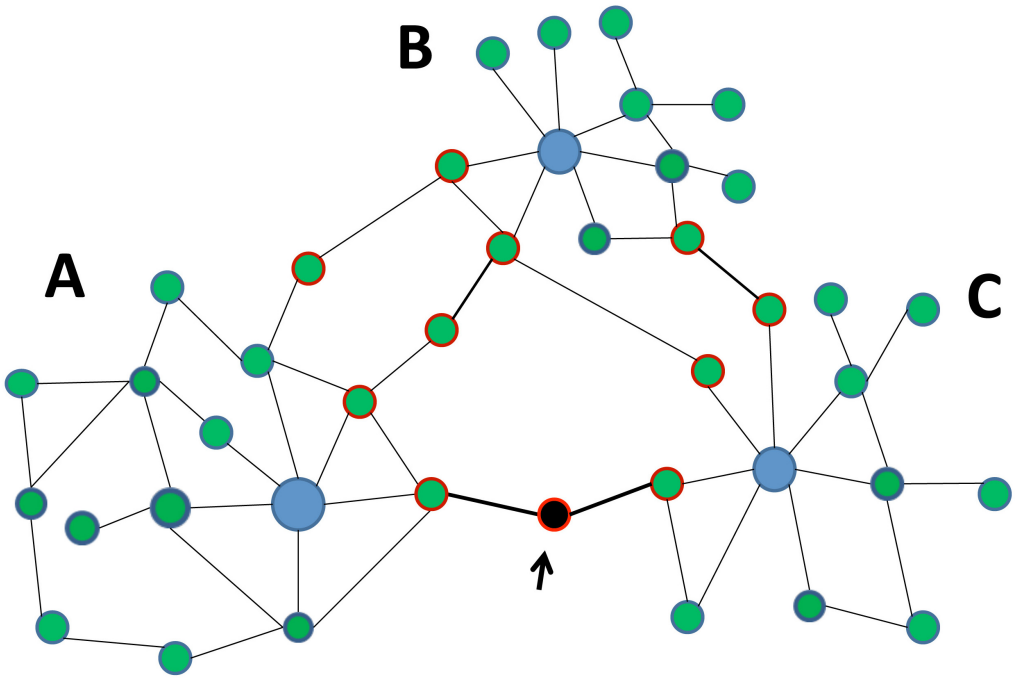

Figure 7: Structural "hole" - the shunt of signals. In normal cells, the signals flow from cluster A to cluster B to cluster C and *vice versa*. Due to the oncogene product (black filled circle) that creates a shortcut between cluster A and cluster C, the cancer cells acquire proliferating advantage over their normal counterparts. The signals in cancer cells will bypass the regulation by cluster B and thus targeting cluster B will be ineffective.

node will react to the stimulus. Stimulus can be single or made up of stimuli from various upstream sources. Some nodes are located at critical points that connect several functional pathways. These nodes are named connector nodes, with which the nodes form a functional network in the cell [Figure 6]. How do we know which connector node has more impact? First we can calculate the density (D) of the functional nodes by using a formula. If N is the number of nodes in the functional network and M is the maximal potential nodal interactions,

$$M = N * (N - 1)/2$$

$$D = N/M$$

The actual impact (I) of the node is

$$I = D/M$$

Centrality has been used by graph theory and network analysis as a measure to identify the most influential factors [63] or connector nodes. However, the most influential connector nodes may not be the ideal targets; key players that connect different clusters of a network may be more important in medicine.

Loners

According to social network theory [63], social relationships are composed of nodes and ties. Nodes are the individual actors within the networks, and ties are the relationship between the actors. There can be

many kinds of ties between the actors (or nodes). In its most simple form, a social network is a map of all of the relevant ties between the nodes being studied [Figure 6]. Nodal interactions are highly diverse and the attributes of individual nodes are less important than their relationships and ties with other actors within the network. People believe a social network does exist in cells [64], which can explain why the same molecule may function differently in a different environment. In contrast to the social network that the impacts are determined by the number of ties, some nodes with less ties in the cell (loners in the network holes) [Figure 7] [65] may be more crucial for the cells to have growth advantage, particularly in cancers. One example is *BCR-ABL* fusion protein [66], a target for imatinib. *PML-RARA* is another such example [67]. Since they are loners in cancer cells, targeting them will cause less toxic effects to normal cells that do not contain them, and thus these loner nodes are usually optimal targets for therapy.

The Balanced Is More Stable

In living cells a new equilibrium is reached every millisecond; the network stability depends on constantly balancing the ever shifting nodal functions. One minor mutation at a noncritical node will not tilt the balance, but when minor mutations accumulate or a mutation affects the critical function of a gene or pathway, the impact may be so huge that the cells either undergo apoptosis, proliferation, or malignant transformation. Apoptosis is usually regulated, occurring as a sacrifice to preserve energy for the normal cells and organs to survive. Malignant changes occur when the cells are out of balance and have passed the "*point of no return*".

Applications

Although the "node" concept has been used in many years and many fields, introducing this concept to life science and medicine will have many practical applications. The concept of functional nodes will help us to examine life and genome systemically, treat disease as a special condition of life, and design optimal targeted therapies for cancer and other diseases. Identifying the "shunting" nodes in the signal transduction network will explain why targeted therapy is sometimes ineffective. Understanding the functional equilibrium of nodes will help us prevent the secondary malignancies when treating cancers. Delineation of the functions of alternative nodes versus decision nodes and their conversions will provide guidance to drug designs for the treatment of diseases. This theory also helps us to understand the limitations of current scientific methods that led to numerous irreproducible research results [68, 69]. New breakthroughs in science are required to revolutionize our current concepts, approaches, and applications in solving the mysteries of gene, life and diseases.

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