Cancer Metastasis Rev
DOI 10.1007/s10555-013-9427-7

Chromosomal instability (CIN): what it is and why it is crucial to cancer evolution

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Abstract Results of various cancer genome sequencing projects have "unexpectedly" challenged the framework of the current somatic gene mutation theory of cancer. The prevalence of diverse genetic heterogeneity observed in cancer questions the strategy of focusing on contributions of individual gene mutations. Much of the genetic heterogeneity in tumors is due to chromosomal instability (CIN), a predominant hallmark of cancer. Multiple molecular mechanisms have been attributed to CIN but unifying these often conflicting mechanisms into one general mechanism has been challenging. In this review, we discuss multiple aspects of CIN including its definitions, methods of measuring, and some common misconceptions. We then apply the genome-based evolutionary theory to propose a general mechanism for CIN to unify the diverse molecular causes. In this new evolutionary framework, CIN represents a system behavior of a stress response with adaptive advantages but also serves as a new potential cause of further destabilization of the genome. Following a brief review about the newly realized functions of chromosomes that defines system inheritance and creates new genomes, we discuss the ultimate importance of CIN in cancer evolution. Finally, a number of confusing issues regarding CIN are explained in light of the evolutionary function of CIN.

Keywords Chromosomal instability (CIN) · Evolutionary mechanism of cancer (EMC) · Genome chaos · Genome heterogeneity · Genome instability · Genome theory · Nonclonal chromosome aberrations (NCCAs) · System inheritance

1 Introduction

The vast majority of cancers display chromosomal alterations [1–5]. This obvious connection should make chromosome instability (CIN) a top priority in cancer research. Unfortunately the ultimate importance of chromosomal aberrations in cancer has been largely ignored in favor of identification of cancer genes [6, 7]. Ironically, the shift from identifying cancer-causing chromosome aberrations to characterizing cancer-causing gene mutations was triggered by the successful identification of the Philadelphia chromosome in chronic myelocytic leukemia (CML) and by the subsequent discovery that the BCR-ABL fusion gene is created by this chromosomal translocation [8]. This shift in focus ushered in the gene cloning era of cancer research and led to the gene-centric concept that has dominated research for the past four decades.

Understanding the rationale/history behind such a shift is important to re-prioritize CIN research. Prior to the era dominated by gene mutation research, there was a period where chromosomal analyses led the field. At that time it was hoped that similar to the case of CML, a signature chromosomal aberration could be identified as the "cause" of each cancer type. However, most tumors have proven to

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Published online: 19 April 2013

possess highly heterogeneous patterns of chromosomal aberrations. Rather than addressing the mechanism and importance of karyotype heterogeneity, research has focused on identifying defined genetic targets. To many, the real "causative" factor(s) of cancer must be identifiable as commonly shared genetic alterations. The ideas of gene mutations being causative factors for cancer and the development of new molecular techniques to characterize cancer genes led to the firm entrenchment of the gene-centric cancer paradigm, which proposes that identification of a handful of causal gene mutations will lead to an understanding of the molecular mechanism of cancer and better cancer treatments by targeting the mutated gene(s) [9–11]. Arguments focusing on cancer genes rather than chromosomes seem obvious: (1) genes are the basis of genetics and cancer is a genetic disease so genes must be the causative factors while chromosomal aberrations must be the result or by-products of cancer gene function. (2) Causality of oncogenes has been demonstrated in model systems and some of these gene mutations are detectable in patients, while chromosomal causality is hard to demonstrate due to the highly diverse patterns that are observed [12]. This has fed the perception that studies characterizing gene mutations or molecular pathways as mechanistic while chromosome based studies are merely descriptive research.

The gene concept has failed to deliver. Cancer genome sequencing projects have sequenced thousands of tumors and demonstrated overwhelmingly that the vast majority of genetic changes are not shared among patients [13–16]. These findings certainly challenge the current framework of cancer theory [17, 18]. In fact, this outcome was predicted at the very beginning of the cancer genome sequencing era, as it was realized that the current somatic gene mutation theory is based on a few exceptional cancer types defined by a linear pattern of progression such as CML [6, 19]. In contrast, most solid tumors progress stochastically, driven by karyotype alteration-mediated punctuated macroevolution [3, 20].

Most cancers display overwhelming genetic heterogeneity [21]. Identification of this heterogeneity, especially at the karyotype level has resulted in increased attention on CIN. Many independent factors previously thought to "cause" cancer have now been linked to CIN through focus on specific molecular mechanisms. Few analyses have focused on karyotypic change as a common mechanism of cancer without being linked to any specific gene defined molecular pathway [20, 22, 23]. Even though there has been a dramatic increase in interest towards CIN, its ultimate importance in somatic evolution has not been fully appreciated [24, 25]. This lack of appreciation has led to much confusion in the field where many "me too" reports claim to have "discovered" key genes for CIN, although among the increasing numbers of linkages between genes and CIN, most of these identified "keys" are minor or rare in the patient population and have limited clinical implications. In this review, we address the following questions: What is CIN? Why is it of ultimate importance in cancer? How should we study it? In particular, we reiterate the importance of CIN in the genome-based evolutionary theory of cancer.

2 A brief review of CIN research

The concept of CIN in cancer was first proposed by Boveri nearly a century ago [26]. Following the successful identification of the Philadelphia chromosome in 1960, cancer cytogeneticists have worked for decades trying to make sense of the diverse karyotype alterations associated with a majority of cancers [27].

Many early CIN studies focused on chromosome instability syndromes such as Ataxia Telangiectasia, Fanconi Anemia, Bloom Syndrome, and Nijmegen Breakage Syndrome [28]. Research has also been directed at the potential link to other cancer types [29] and cancer evolution [30]. The idea of genetic instability including CIN has also been used to explain the mutator phenotype hypothesis [31]. Though there were some established molecular mechanisms of genomic instability [32], it was Vogelstein's group who highlighted this issue by classifying genetic instability into CIN and micro-satellite instability, reinforcing the idea that most colon cancers are indeed chromosomally unstable [33]. Following a series of reports from Vogelstein's group, scattered reports began to suggest that CIN was an important causative factor in most cancers [34]. For example, telomere dysfunction was linked to promoting nonreciprocal translocations in epithelial cancers [35], nonhomologous end-joining (NHEJ) was linked to genomic stability and the suppression of chromosome translocations [36], Histone H2AX was linked to suppression of oncogenic translocations [37], ATM was linked to suppression of aneuploidy and subsequent tumor formation [38], germline BUBR1 mutations were linked to aneuploidy and cancer predisposition [39], and various new types of chromosomal aberration including different types of nonclonal chromosome aberrations (NCCAs) were identified and linked to cancer [3]. Together, these exciting reports support the assertion that aneuploidy is more important than gene mutations in cancer [40, 41].

As a reflection of the changing attitudes towards chromosomal aberrations in cancer, a few popular journals have published reviews, opinions, and debates on the importance of CIN in solid tumors [34, 42–44], including experimental and theoretical evidence on the role CIN plays in the initiation of cancer [45], and the possible link between CIN and aneuploidy [46]. The issue of whether NCCAs are important has also been raised; as in stark contrast to hematological malignancies, recurrent chromosomal aberrations are rare in solid tumors [2]. It is worth mentioning that an influential piece in the Scientific American garnered needed attention

by pointing out the promise of the chromosome based cancer theory and the limitations of the popular gene mutation theory [47].

There are two major advances that underscore the importance of CIN. The first is the use of NCCAs to measure CIN and the identification and characterization of new types of chromosomal aberrations that contribute to CIN [3, 20, 48, 49]. To date CIN studies have focused primarily on the identification of clonal chromosome aberrations (CCAs) as traditionally, NCCAs are considered nonsignificant genetic noise [2, 20, 50]. Analysis of chromosomal aberrations in a model of cancer evolution led to the realization that seemingly random NCCAs actually reflect system instability and drive cancer evolution by increasing population diversity. A series of experiments further demonstrated that CIN (elevated NCCAs) increases multiple times during cancer progression regardless of which "causative" factor is associated with that progression. Therefore, NCCAs best represent CIN and CCAs actually represent chromosomal stability [3, 4, 48, 49]. This finding is significant, as it clarifies confusion as to which chromosomal aberration types should be used to measure genome stability or instability. This advancement not only links different genetic, epigenetic and environmental factors to CIN, but also demonstrates that any molecular mechanism can act as a system stress by contributing to the evolutionary mechanism of cancer (EMC; for more, see section below). The EMC unifies the diverse molecular mechanisms of cancer and stochastic genome aberrations.

The second major advance stems from the recent cancer genome sequencing projects intended to identify shared common gene mutations in an unbiased manner [6, 13–16]. These studies have revealed extensive mutational heterogeneity between tumors. In particular, these studies clearly show that the vast majority of cancer cases contain complex chromosome alterations. Though these sequencing papers contain limited discussion on complex genome rearrangements, their message is so striking that it has altered popular views of the cancer genome, as many had previously thought that cancer cells displayed normal or near-normal karyotypes and that key gene mutations drove cancer. The prevalence of these drastically altered cancer genomes underscores the new urgency for CIN studies.

The rapid growth of CIN-related literature documents the general acceptance of CIN's importance. Various models have been proposed to explain the mechanism of CIN including: how changing alterations of a specific oncogene or tumor suppressor leads to structural or segmental chromosomal change [51, 52]; determining the topological features of nuclei associated with chromosomal rearrangements [5, 53]; determining how replication stress affects genomic integrity [54]; and explaining the common mechanism of aneuploidy [55–57]. Obviously, current major efforts still focus on individual gene or pathway-based mechanisms as they link various cancer genes to chromosome reorganization including fusion genes, gene deletions, regulatory change, and dosage effects.

Realization of CIN's importance raises the question of what is the common mechanism of CIN. In order to link the multitude of diverse molecular mechanisms of CIN, a new framework called the genome theory has been introduced [22, 58]. The genome theory emphasizes the importance of the karyotype defining the biological system, how stress leads to adaptation through karyotypic alteration, and how genome heterogeneity defines the patterns of cancer evolution [7, 20, 59–61]. This new synthesis reveals the evolutionary meaning of CIN, which not only answers why CIN is ultimately more important than other individual molecular mechanisms, but also unifies diverse genetic changes within an evolutionary framework.

3 Definition and challenges of measuring CIN

The definition of CIN seems straightforward. CIN is the rate (cell to cell variability) of changed karyotypes of a given cell population. CIN can be classified as structural CIN or numerical CIN [5]. Numerical CIN is determined by gain or loss of whole chromosomes or fractions of chromosomes (aneuploidy), while structural CIN is determined by structural NCCAs. In a normal (nontumorgenic) cell population, CIN results in an increased proportion of cells with aneuploidy and/or structurally altered chromosomes. In a cancer cell population, CIN generates an altered karyotype landscape by increasing or reducing NCCAs. In practice, however, CIN is difficult to measure. First, most current high-throughput molecular methods are based on average population profiles and cannot detect cell to cell variability. Therefore to study cell to cell variability tedious molecular cytogenetic analyses are required [3–5, 62, 63]. However, challenges exist in accessing enough mitotic cells in cancer tissues and the identification of diverse chromosomal aberration types. Single cell CGH arrays or single cell sequencing can avoid the need of preparing mitotic chromosomes, but a large number of cells are needed to measure CIN, making these impractical on a routine basis. In addition, many chromosomal aberrations are still difficult or impossible to detect without cytogenetic methods. Furthermore, to truly measure the rate of chromosomal change, chromosome aberrations must be measured over time in order to watch evolution in action. This approach is not common in CIN analysis.

Perhaps the biggest challenge to measuring structural CIN is the diversity of chromosomal aberrations. In addition to well-known structural aberrations including simple translocations, complex duplications, deletions, double-minute

chromosomes, homogeneously staining regions, multicentric chromosomes, ring chromosomes, lagging chromosomes, sticky chromosomes, small supernumerary marker chromosomes, and multiradial chromosomes, there are many currently unclassified forms of aberrations including defective mitotic figures, chromosome fragmentation, and genome chaos [3, 4, 7, 60, 61, 64]. Inclusion of as many of these aberrations (many of which have so far been ignored) increases the power of CIN analysis.

Currently, there also is no standard formula to integrate structural and numerical CIN quantitatively, although structural NCCAs seem to more effectively predict tumorigenicity than numerical NCCAs [23]. Structural NCCAs are likely to be even more predictive when massive genome reorganization (structural genome chaos) occurs. The overall impact of structural and numerical NCCAs can be dependent on the system in which they are measured. For example in a mouse *in vitro* transformation model, we found that chromosomal translocations are often later stage events while aneuploidy occurs throughout [23]. Increased dynamics occur at multiple levels of genetic organization including the sequence and transcriptome levels in unstable genomes [65]. Gross chromosomal translocations are often associated with high levels of cryptic rearrangements at the DNA level [66]. Future research is needed to compare and integrate the contributions of different types of CIN with other genetic/epigenetic alterations in cancer to increase the predictive power of CIN [4, 7]. Efforts are also needed to determine how the coexistence of CCAs and NCCAs contribute to cancer evolution. Subpopulations with CCAs exist in most tumors but these cells often have unique NCCAs as well.

A common error in current CIN studies is confusing CCAs for CIN. Historically, chromosome aberration studies have focused on CCAs and disregarded NCCAs [2, 20, 50]. This bias also exists methodologically as molecular methods such as arrays only measure the average value of a population. These technologies retain CCAs and "wash-out" NCCAs. As we previously pointed out, CCAs in fact are an indicator of relative stability. The CIN profiles of cell populations must be based on single cell methodologies preferably cytogenetic analysis.

4 Unify the unlimited causative factors of CIN

CIN is the driver of cancer progression, and the identification of the mechanism of CIN is highly significant. Ever increasing diverse mechanisms have been linked to CIN. Determination of the relative importance of each mechanism is difficult as there are so many potential mechanisms most of which are not highly prevalent within patient populations.

The diverse CIN mechanisms can be classified into two major types (Table 1): type I includes mechanisms that are directly linked to the maintenance of genome integrity throughout the chromosomal cycle including the chromosomal machinery, checkpoints, and repair systems. Each step of the chromosome cycle is impacted by multiple pathways and involves many genes. For example, in just the segregation step defects in any part of the entire process can lead to CIN including defects of the condensation, spindle checkpoint, kinetochore-microtubule attachments, sister chromatid cohesion, centrosome duplication and bipolar spindle assembly [64, 67]. Other parts of the chromosome cycle that are directly implicated in CIN include DNA replication, chromosome condensation/decondensation, and cytokinesis.

Type I mechanisms are often associated with chromosome instability syndromes and are straightforward due to the direct "molecular causative relationship" between identified factors and CIN. However, mutations to type I genes are rare and they do not explain sporadic cancer. For instance, germline mutation of spindle checkpoint gene BUB1B is linked to mosaic variegated aneuploidy and predisposes to various types of cancer [39]. However, this mutation is rare in human tumors. In fact, whole cancer genome sequencing so far has failed to identify novel putative caretaker genes that are frequently mutated in cancer, and only 3–31 % of untreated sporadic human cancers have one or more mutations of a caretaker gene [54]. Of note here, there is a huge gap between identification of gene mutations and the true cause of cancer. For example, the recent 1000 Genomes Project revealed that normal individuals have on average 250–300 loss-of-function mutations, and 50–100 of these variants have been implicated in inherited diseases. On the other hand, "driver mutations" number is estimated to be a fewer than 100 for many cancer types [14, 68, 69]. Thus, even having a type I CIN mutation does not make cancer certain. Though anecdotal, Dr. James Watson is an interesting case of the disparity between specific mutations and cancer. He has 310 gene mutations including DNA repair genes linked to CIN and cancer and at the age of 84 is cancer free. This illustrates the uncertainty of predicting cancer based on gene mutations that are directly linked to CIN.

Type II CIN mechanisms are those that do not have a direct molecular causative explanation, however, they clearly are linked to a CIN phenotype under certain conditions. Since type I mechanisms occur infrequently in sporadic cancers, it is reasonable to suggest that type II mechanisms are more common (Fig. 1). Many have attempted to link type II mechanisms to known gene or pathway defects associated with type I mechanisms. It is challenging to gauge how reliable these published links are, as reports often conflict. More importantly, whole genome expression studies have illustrated that any specific link is connected with so many other links that complex relationships often cannot be directly pinpointed without reducing the complexity of the cellular system and therefore the relevance of a given complex relationship [7]. Type II CIN mechanisms

Table 1 Two types of molecular mechanisms that lead to chromosome instability

Type I: the maintenance of genomic integrity
Maintenance of the chromosomal cycle (pathways/machinery that are responsible for DNA replication, chromosomal condensation, chromosomal segregation, and chromosomal de-condensation)
Maintenance of chromosome structure (integrity of telomeres; centromeres; retrotransposons; chromosomal hotspots - fragile sites and highly transcribed DNA sequences, and the 3-D chromatin topology)
Maintenance of DNA methylation status
Repair processes of any internal or induced errors occurring from above mentioned processes
Type II: produce various stress that increase the system dynamics
General system dynamics response caused by various factors (infection, metabolic status, epigenetic alterations induced by environments, aging, physiological stress, toxins, pollution, etc.)
Multiple levels of instability (depending on the degree of stress)
Responses with adaptive and survival advantages (immunoglobulin diversification, liver cell adaptation, and genome chaos occurring under crisis)
Major contributing factors to stochastic cancer evolution that are less specific (occurring through the formation of all types of genetic/nongenetic alterations that ultimately lead to new genome defined systems generated by evolutionary selection)

are frequently linked to nongenetic factors such as the micro-environment and physiological processes (aging, hormones, inflammation, and metabolic status), which importantly integrates the impact of the environment into the CIN-cancer framework. Of note, previous publications suggested that some oncogenes do not promote CIN. However, these reports either did not directly examine the issue of CIN, or only monitored CIN using clonal aberrations. Many of these studies can be explained by the type II CIN mechanism.

The recently established EMC offers a framework unifying the common mechanisms of CIN, especially type II CIN [23, 25, 70]. There are three key preconditions for cancer evolution to take place: (a) there must be variation within the cellular population, (b) the variation must be heritable, and (c) the variation must affect survival and/or reproduction of altered cells [71]. It is also important to know that there are two phases of cancer evolution where the macro evolutionary phase plays an essential role in cancer progression as multiple new genome defined systems are needed to overcome multiple levels of system constraint [6, 20].

According to the EMC, a multitude of molecular mechanisms contribute to cancer and each is linked to four key components of the evolutionary process. These components include (1) internal and external stress which generate variation, (2) elevated genetic and nongenetic variation and resultant adaptation, (3) genome replacement-based macro-evolution that ensures a point of no return, and (4) loss of system homeostasis and the multilevel system constraints that ensure homeostasis which serve as a major constraint preventing cancer evolution. Stress induced genome instability drives dynamics/variation/evolution, while the multiple levels of system constraint represent the opposing forces of homeostasis/conservation [7]. The key to tipping the balance is increasing heritable changes.

The common link between the diverse type II mechanisms is that they are all involved in the cellular system's response to stress increasing heritable changes. During cancer progression, many diverse individual mechanisms (including type I mechanisms) function in response to stress to a system, inducing CIN [20]. The karyotype dynamics of CIN lead to adaptation to stress [61, 72]. Under normal physiological conditions, the cellular system handles stress by expending energy without the need for additional genetic/epigenetic changes. However, when stress is persistent and particularly of a high degree, lower-level genetic/epigenetic alterations become required for survival [7]. These alterations provide adaptation to stress. As an example, liver cells become polyploid and aneuploid in response to stress in order to preserve normal function [73, 74]. The adaptability instilled by CIN is important, as it explains the complexity of biological alterations and their implications to the cellular system. Unfortunately once system stability is negated in favor of adaptation, an array of bioprocesses will have a higher chance of generating errors. These errors provide potential of generating a cascade of further change and associated problems. Regardless of the problem that induces such a complex relationship, the key to cancer progression is CIN-generated genome diversity that drives somatic evolution.

The "stress–CIN–cancer–evolution relationship" explains the unpredictability of NCCAs and why many divergent molecular mechanisms can lead to CIN, and why CIN leads to diverse bioreactions (each NCCA creates a new genome-defined system with specific pathways) (Fig. 1). This relationship also explains why a large number of gene mutations are detected in a specific tumor yet have little overlap with other tumors of the same type. More importantly, this relationship links many nongenetic environmental factors to CIN. Given the unlimited number of mechanisms leading to CIN and the fact that most of these are rare within a patient population, continuing efforts attempting to link

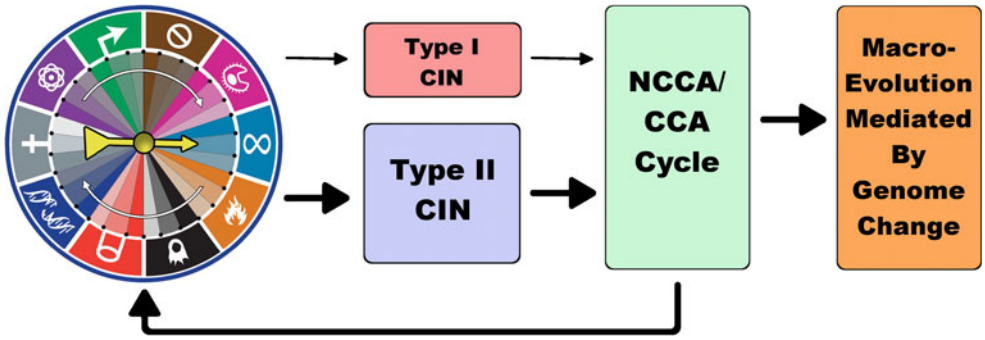

Fig. 1 Diagram illustrating the relationship among stress, diverse individual molecular mechanisms of cancer, CIN, and stochastic genome change-mediated cancer evolution. The hallmarks of cancer (adapted from [136]) was used to represent different pathways linked to cancer. Stress is the motor that turns the pathway wheel. Selection of a given pathway as a mechanism of cancer progression is represented by the arrow which selects a pathway based on probability. Individual pathways can directly compromise genome integrity (type I) or indirectly jeopardize genome integrity through general stress (type II). Both types I and II CIN are linked to elevated NCCA frequency. Stress-induced CIN is the key generator of evolutionary potential leading to macro-cancer evolution

various genes/pathways/biological processes/environmental factors to CIN should not be a priority.

Caution is required when interpreting the literature. First, parts of the cell/chromosome cycle which can impact chromosome stability have been overlooked [3, 64]. Genetic instability studies have long focused on the DNA replication/repair process and more recently chromosome segregation has received increased attention, but research of chromosome condensation and de-condensation are still overlooked. Second, most publications focus on linking specific known molecular pathways to CIN, but there is a complicated relationship between different types of CIN mechanisms. For example, DNA methylation status has been extensively studied in cancer genes, but changes to methylation also initiate activation of retrotransposons which can alter the topology of the genome or the interactive relationship among individual genes [75] or more importantly serve as a stress that can be classified as a type II mechanism. Third, most publications have emphasized molecular certainty when studying causative relationships. Under high stress levels, however, adaptation through stochastic chromosome change is the rule rather than the exception, as it was illustrated by dynamic NCCA/CCA relationship under stress and during cancer evolution [20, 62]. For example, the appearance of double-stranded breaks (DSBs) activates multiple specific mechanisms meant to deal with the problem, but these diverse mechanisms are stochastically linked to the repair process depending on the context in which the breaks occur. The existent stochastic relationship between DSBs, repair mechanisms and types of chromosomal rearrangements is best illustrated by the following example. There are at least six repair pathways that can lead to chromosomal rearrangement [76]: single-strand annealing can lead to interstitial deletions; Break-induced replication can lead to nonreciprocal translocations and interstitial deletion/inversions; standard DSB repair, synthesis-dependent strand annealing, and NHEJ can lead to reciprocal translocations and interstitial deletions/duplications/deletions; and telomere addition and breakage–bridge fusion can lead to terminal deletions and translocations. Such complexity negates the predictive ability of any specific individual mechanism in practice.

5 The "new" function of chromosomes

Since CIN leads to chromosome changes, understanding the function of chromosomes is central to understanding CIN. According to genetics 101, the function of chromosomes is to serve as a vehicle to ensure faithful transmission of genetic material between mother and daughter cells both in germline and somatic cells. Gene defects that impact on genome integrity will lead to CIN. Therefore, CIN is the consequence of gene mutation, and a late event compared with initial gene mutations. Furthermore, many cells with altered chromosomes are nonviable and will be eliminated. Finally, most nonrecurrent chromosomal aberrations are meaningless and function as genetic noise, and only those that expand clonally are of significance (such as the Philadelphia chromosome).

It turns out that the chromosome is not just a vehicle of genes but the key organizer of genetic information. The chromosome is the determinant of genome context which is the gene/DNA sequence content plus the genomic topology [7, 22, 24, 58]. Any chromosomal changes that alter the karyotypic system change the context of the information the system carries, impacting expression of many genes and more significantly altering the cellular system. The impact of genome change is therefore more powerful than any individual gene mutation [6, 7]. Significantly, the level of stochastic chromosome alterations is indicative of the genome dynamics of a cell population. NCCAs thus have profound evolutionary impact and chromosomal alterations

are not necessarily late events caused by gene mutations, though this is one possibility. Furthermore, the level of stochastic chromosome alterations is indicative of the genome dynamics of a cell population.

The development of the genome theory of evolution prompted the above realization [7, 58]. The genome theory resulted from a number of diverse and seemingly isolated concepts/facts/observations including: first, genes code for parts (such as a specific protein or RNA or regulatory element) while the chromosomal defined genome provides the information of the architecture of the system [7, 22, 58]; second, gene sequences are preserved by the fidelity of replication and other repair mechanisms, while the conservation of chromosomes is preserved by sexual reproduction (chromosome pairing during meiosis and additional filters eliminate significant chromosomal changes and conserve the identity of a species) [77–80]; third, genomic analyses show that during evolutionary history, major lineage changes are commonly associated with genome-level changes (genome duplication, chromosomal reorganization, etc.). Each species has a unique karyotype while species-specific genes are uncommon; fourth, in cancer, genome-level changes drive macroevolution even though the gene content remains similar. The vast majority of cancers display stress-induced altered karyotypes; fifth, genomic topological changes can lead to new traits. For instance, reciprocal chromosomal translocations impact the fitness of *Saccharomyces cerevisiae*, supporting a model of fixation by natural selection of reciprocal translocations in *Saccharomyces* species [81]. Recently, an observation of evolution in action in a continuous culture experiment has shown that a novel trait can evolve through genomic rearrangement and amplification of a few pre-existing genes, supporting the importance of the genomic topology [82]. Furthermore, loss of function due to specific deletion can be restored by a changing chromosome status [83]. Together, the message is loud and clear, chromosome defined genome context represents the highest level of genetic organization, and it is the karyotype that controls the system inheritance (in contrast, genes only code for the "parts inheritance"). The main function of chromosomes is to maintain the system inheritance by providing genomic topology, and biological properties emerge from gene and DNA sequence interactions within the context of the genomic topology. There are increasing suggestions that cancers with different or unique karyotypes should be treated as different systems or species [4, 7, 70, 84, 85].

As a result of altering the genomic system, aneuploidy and translocations drastically change the dynamics of the transcriptome and the complex system of interactions that derive from the transcriptome [24, 65, 86]. These chromosomal changes are not limited to only impacting some individual genes, but more fundamentally, they alter the entire platform of gene interaction by altering the genomic topology. Clearly, with this new understanding of the function of chromosomes, CIN is an important issue in cancer. Interestingly, CIN also contributes to the inherited heterogeneity, yet another level of inheritance linked to cancer evolution (in addition to genome-defined system inheritance).

6 The ultimate importance of CIN in cancer evolution

To understand why CIN is the most important issue in cancer, in addition to understanding the new genetic functions of chromosomes, appreciation of cancer as a somatic evolutionary process, for which CIN is essential, is required [20, 30, 71, 87].

Treating cancer as an evolutionary problem is not new. Nowell's concept of clonal evolution, largely based on understanding CML, originated the stepwise evolutionary viewpoint that dominates the field. Stepwise evolution is attractive as it offers the possibility of a fixed targetable early marker useful for both diagnosis and treatment. However decades of research and more recently cancer genome sequencing projects have failed to identify stepwise patterns. In contrast CIN is shared across tumors. Observation of the patterns of karyotype evolution throughout the entire process of *in vitro* immortalization and acquisition of drug resistance, revealed two distinct phases of cancer evolution: punctuated and stepwise phases [6, 20, 70]. CIN dominates during the punctuated phase where NCCAs are common. During the punctuated phase karyotype replacement dominates. Recently, punctuated evolution has also been confirmed at the gene level by sequencing the cancer genomes of single cells [88]. In contrast to the punctuated phase, within the stepwise Darwinian phase, CIN is low and most cells share similar genomes. During the Darwinian phase, clonal evolution is evident as gradual genome re-organization is traced similar to the karyotypic patterns observed in chronic phase CML patients.

Observation of the two phases of cancer evolution and associated follow up studies were essential to understand the patterns of somatic cell evolution in cancer. First, there is no simple or linear clonal evolution in most cancers [20, 25, 88]. Even in CML, once the blast crisis stage occurs, punctuated patterns of evolution dominate [89].

Second, CIN plays an important role within the punctuated phase where stochastic genome re-organization dominates [4, 48, 49]. In this phase (including blast phase CML), there is no stepwise accumulation of genetic changes, and even the most successful targeting drugs like imatinib are no longer effective due to population heterogeneity caused by drastic genome evolution [89].

Third, the mechanisms behind the punctuated and stepwise phases of evolution differ. Based on somatic cell and organismal evolutionary patterns, we have hypothesized that genome re-organization drives the punctuated phase while gene mutations or epigenetic alterations accumulate within the stepwise phase [7, 22, 58].

Fourth, switches from one phase to the other are dependent on CIN. Fit cells with stable genomes are able to expand clonally and enter into the stable stepwise phase. Under high levels of stress such as telomere shortening that can cause cellular crisis or drug treatment-induced genome chaos, CIN is induced, pushing cells from the stepwise phase into the punctuated phase [6, 20, 60, 61]. High levels of CIN increase both the frequency of cell death and karyotype replacement until a cell with stable, viable, and advantageous karyotype forms a stable population. In most solid tumors, somatic cells undergo multiple cycles of the two phases of evolution as they transition through immortalization, transformation, tumor formation, metastasis, and drug resistance [70, 90, 91].

Fifth, the punctuated and stepwise phases represent macro- and microevolution respectively. CIN is the key driver of macroevolution. During macroevolution, genome alterations change the system and in contrast, during microevolution gene changes modify the system. Genome reorganization must occur for drastic changes to the new system to occur, as it is the most effective means to alter the transcriptome to meet the requirements of evolution [22, 24, 65].

Sixth, the degree of CIN (NCCA frequency) is linked to tumorigenicity and drug resistance [6, 23]. Furthermore, the inherited heterogeneity amplifies the evolutionary potential for drug resistance (Abdallah et al, unpublished data). Increased CIN is typically associated with increased survival potential, while reduced CIN (CCAs) increases growth [4].

Seventh, the CIN-NCCA-CCA relationship explains the multiple level adaptive landscape model. According to this model [22], to cross the global landscape, stress induced CIN is essential as its induced karyotype dynamics generate new systems (NCCAs) that can form new CCAs if they are fit for that landscape or can produce new NCCAs and associated landscapes. During key stages of cancer progression such as immortalization or acquisition of drug resistance, the "winning" karyotypes emerge following multiple runs of evolution, and each run requires different karyotypes. Previously, most adaptive landscape models focused solely on the local scale where gene or nongenetic instability dominates [92, 93].

Synthesis of the above information within the framework of the genome theory and the EMC which ascribes new functions to the chromosomes (karyotype) and in light of this, it should be obvious that CIN drives cancer evolution. For cancer evolution to be successful, high degrees of genetic variation, particularly genome-level variation, are essential. CIN alters system inheritance by altering the transcriptome, therefore CIN is a pre-condition for cancer evolution. In contrast, gene and epigenetic level alterations, in spite of their importance in microevolution, are unable to break through the multiple levels of system constraint (genome, tissue, organ, and individual overall homeostasis including immune-system constraints), and only when these lower levels of genetic/nongenetic alteration trigger CIN will this lead to macrocellular evolution.

Figure 1 also illustrates such evolutionary mechanism. Sufficient stress (potentially any errors or even normal processes associated with individual molecular mechanisms such as aging or metabolic processes) can trigger various genetic changes including NCCAs. Any NCCAs are likely associated with lower-level genetic alterations. In contrast, lower levels of genetic change might not lead to changes at the higher genome level. Increased NCCAs frequency increases evolutionary potential. However, most newly formed NCCAs do not survive long term due to competitive disadvantages and system constraints. Nondetrimental NCCAs will continue to exist, remaining neutral. A very small percentage of NCCAs with competitive advantages will provide an advantage and go on to form CCAs, entering the stepwise evolutionary phase and occupying different attractors within the landscape (Fig. 2). While each CCA can be linked to a specific molecular pathway at a given time, the process of getting to that point is stochastic limiting the predictability of the consequences of CIN. Due to the constantly changing selection forces, most CCAs are transitional and will be eliminated or change to other NCCAs or CCAs. Persistence of stress forces previously formed CCAs and NCCAs (evolutionary reservoirs) to enter another NCCA/CCA cycle further changing the genomic landscape, increasing the probability of escaping the multiple levels of system constraint [6, 7, 58]. Increasing the number of cycles increases the probability of cancer formation, as cancer is a genomic disease of probability.

7 The evolutionary implications of CIN solve many puzzles

The evolutionary implications of CIN address and/or solve many questions of cancer. The following are some examples:

(a) What is the common cause of cancer evolution and does determining every diverse factor that contributes to cancer better our ability to fight it? There has been intense interest and debate on the causes and consequences of CIN [20, 47, 56, 94–97]. One frequent comment regarding the ubiquity of chromosome aberrations and the diversity of gene mutations detected during both the process of cancer formation and in

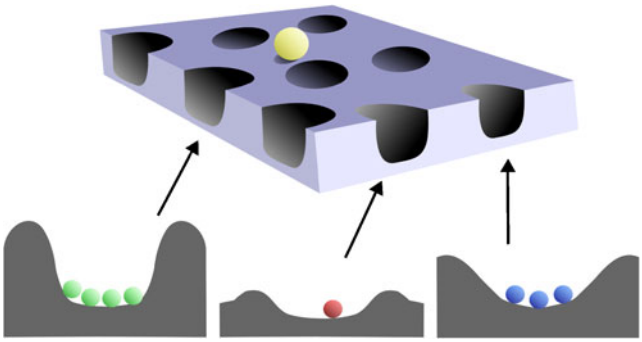

Fig. 2 The NCCA/CCA relationship within the cancer landscape. Cells are represented by *spheres* and each *color* symbolizes a genome that is unique to that color. The *single yellow sphere* represents the spectrum of NCCAs. If a NCCA encounters the right attractor landscape (represented here by different *holes*), that NCCA will expand clonally forming a CCA (*bottom row*)

established tumors is, "the cancer genome certainly displays diverse genetic alterations, but most of these changes are only correlation not causation. We need to identify the real drivers and/or causative factors". Identification and targeting of key causative factors of cancer is the current cancer paradigm. Different "parts centric" theories have come and gone. These "fashions" so far have failed to establish a unified molecular model of cancer, despite high hopes and convincing publications based on specific cases [6, 98]. Most of these theories, where a clear predisposing mutation can be identified as the initial genetic causation, explain only a fraction of all cancers. It was thought that these exceptional cases could provide knowledge central to all cancers and that successful treatment strategies based on these exceptional cases could be generalized to the broader patient population [99]. This promise has not been fulfilled as biological heterogeneity impedes the generalization of exceptions [6, 7, 20, 21]. Gene mutations responsible for inherited cancers are unaffected in most sporadic cancers, and cancer genome sequencing projects have not uncovered previously unknown high-frequency mutations [100]. New efforts to identify specific causative agents now focus on epigenetic variation including diverse noncoding RNAs that can share mechanisms with previously known gene mutations [101, 102]. Though some correlations can be found, similar to gene mutations, these lower-level alterations will not function as the principal causative factors in cancer. If gene mutations have limited implications in most tumors, implications of other ancillary factors linked to gene mutations likely will be even more limited. The EMC led to the realization that there is no universal cancer mechanism at the lower molecular level. The EMC reveals the importance of CIN as it unifies all individual molecular or biological mechanisms. Despite the highly diverse genome alterations caused by diverse factors, the common cause of these genome dynamics is system stress, and the common elevated genome-level alterations of CIN ensure the success of macroevolution. Therefore, focusing on individual mechanisms is of very limited value as the majority of them occur in low frequencies within patient populations. Individual mechanisms are readily replaced during the evolutionary process when stress causes system instability (mostly through CIN). In a sense, CIN is selected for by cancer cells. The argument exists that genetic instability is not a requirement for cancer evolution, as natural selection drives cancer [97]. However, as extensively discussed, chromosomal changes are essential for macroevolution to occur. Macroevolution cannot simply be explained as a result of natural selection and thus CIN is a necessary means for cancer evolution to be successful. Superficially, cancer evolution seems to select for growth advantages but fundamentally it selects for genomic adaptability to form new genome defined systems capable of overcoming multiple constraints. This is why CIN is more important than the ability to proliferate, as proliferation is just a feature (particularly during the late stages) of the selected system [7]. There is a major concern that the current cancer framework focuses on identifying specific mechanisms without addressing the "first principles" [103]. Evaluation of whether the EMC represents such a first principle, particularly with regard to how different genetic levels are responsible for the different patterns (micro or macro) of cancer evolution. Though most cancer researchers consider genetics as a first principle of cancer biology [103], it is not understood that system inheritance is a function of the genome rather than the sum of the genes [22, 58, 70]. In addition, synthesis of this principle is impeded by the high level of unpredictability in genome-level evolution. Clearly, the complex relationship between CIN and diverse molecular mechanisms within the dynamic time window of cancer evolution is quite confusing.

(b) What role does CIN play in somatic cell and organismal evolution? The presence of CIN in somatic cells is puzzling as gene mutations that reduce genome integrity should be selected against. A common viewpoint is that selection pressure against these genes wanes with age especially after reproductive age. In contrast, the genome theory offers a very different explanation. Since there is a major separation between genome reorganization-defined macroevolution and gene mutation-mediated microevolution, gene mutations can come and go so long as the genome makeup is preserved by sexual reproduction [58, 77, 79].

Therefore, short term adaptation of individuals requires gene dynamic changes, while the long-term existence of a species depends on conservation of the genome. To maximize both evolutionary dynamics and maintain constraint, there is a layer of separation that exists between the germline and somatic cells. The sexual filter ensures (purifies) germline genome integrity while diversity of somatic cells is allowable and enables adaptation [7, 58, 73, 74]. This could explain why genome alterations are frequently detected in highly metabolic tissue, in aged tissue or tissue under stress. Genome alteration increases the cancer evolutionary potential, but does not impact the germline genome. In a sense, cancer evolution potential is the price we pay for adaptability. CIN also explains why somatic cell macroevolution requires a much shorter time period compared with organismal macroevolution. Somatic cells lack sexual reproduction and do not have an effective mechanism to eliminate altered genomes, allowing multiple rounds of macroevolution (similar to speciation events) to occur within a few decades or less. In contrast, the vast majority of genome alterations that occur during reproduction are nonviable and cannot contribute to speciation. Therefore, speciation events typically can only be observed over a much longer time period [7, 58, 77, 79, 80].

(c) Why can CIN both promote and suppress cancer evolution? CIN is the major diversifying force of macroevolution and it is context dependent. In healthy somatic cells, CIN can promote the diversity needed for adaptation with the drawback that it increases the probability of cancer. From a cancer cell point of view, all change that triggers somatic cell evolution is good until the tumor is established. Whether or not additional CIN will promote or suppress cancer evolution in experimental systems depends on the system examined. In many models, the introduced CIN will promote cancer evolution in general as it increases the probability that one or more cells will escape selection pressures of multiple constraints. In contrast, in cancer models driven by strong cancer promoters that progress rapidly additional CIN will often slow the process. The establishment of the two phases of evolution model explains why. Introducing CIN during a different phase will lead to very different responses. During the stepwise phase, additional CIN disrupts the already selected clonal population by interfering with clonal expansion (4). Destabilizing the established CCAs negates any growth advantage that stemmed from the initial CCA and requires a new NCCA with a selection advantage to spread throughout the population, slowing down evolution. Given enough time, the newly formed CCAs could continue cancer evolution (only occasionally, new and more powerful CCAs may arise speeding evolution). Introducing further CIN during the punctuated phase will result in increased instability and most of these unstable cells will not be viable, but this highly stressful condition also promotes genome chaos increasing the probability of emergence of powerful CCAs. Thus, CIN can paradoxically suppress and promote cancer evolution. Clearly, the short term and long term advantages/disadvantages constantly change. This prediction is supported by experiments illustrating that aneuploidy can function both as an instigator and inhibitor of tumorigenesis [104]. For example, cells and mice with reduced levels of centromere-linked motor protein CENP-E have been used to study the relationship between aneuploidy, CIN and tumorigenesis. In addition to demonstrating that an increased rate of aneuploidy drives tumors in aged animals, CIN was surprisingly linked to tumor suppression when other dominant mechanisms of cancer were present. The two model systems in this study were 7,12-dimethylbenz[a] anthracene (a potent carcinogen) treatment and p19/ARF deletion that potently induces tumors in mice by 2 months of age. These models induce a strong, dominant cancer pathway. Additional aneuploidy then functions as a system destabilizer that slows the evolutionary process. Interestingly, there were indeed exceptions as tumors in a few animals grew even faster with aneuploidy. The above observations have been thought to be a paradox of aneuploidy [105, 106]. However, they are reconciled by using the evolutionary concept described earlier. As to the question of why aneuploidy alone in normal physiological conditions is not sufficient for cancer to occur (leukemia is more rare in children with Down syndrome for instance), the answer might not have to do with the aneuploidy itself, but whether or not there is competition between cells with aneuploidy and surrounding cell populations that do not exhibit this aneuploidy. Though altered from normal, the Down syndrome genome is relatively stable. Recently, the issue of somatic chimerism is attracting increasing attention, as the high levels of genome alterations are commonly observed at the somatic cell level in patients and seemingly normal individuals [7, 107–110]. It thus would be interesting to study the instability of Down's individuals with chimeric genomes. The fact that aneuploidy generates significant stress on the cell should be considered in such a context, as evolutionary potential derives from cellular variation-mediated selection [111, 112]. The same argument regarding the paradoxical functions of CIN in cancer evolution applies to many other factors such as the function of a specific gene or specific drug treatment, which even questions the rationale of classifying

"cancer genes" into oncogenes or tumor suppressors, as these functions can change with a different context [6, 7, 67]. It also explains why chromosomally unstable tumors may initially be more sensitive to treatment drugs, but paradoxically more commonly acquire drug resistance. From a molecular mechanistic point of view, targeting cancer is a simple principle. From an evolutionary point of view, which more faithfully follows real world situations, the consequences of targeting are not predictable.

(d) Why is CIN more important than other types of instability in cancer evolution? There are many types of system instability related to cancer evolution. Current molecular research focuses on the instabilities at the cellular level, in particular genetic and nongenetic instabilities [33, 113]. Genetic instability is often referred to the nucleotide instability (NIN), microsatellite instability (MIN or MSI), CIN, and whole genome loss of heterogeneity [114, 115]. Nongenetic instability including epigenetic instability has also become increasingly popular [116, 117]. Instability of chromatin topology as well as heterochromatin instability is now under investigation [118, 119]. We recommend using genomic instability or genome instability to replace the term genetic instability when referring to NIN, MSI, or CIN, as genetics traditionally focuses on individual genes while genomics focuses on the genome [7]. CIN and karyotype instability are interchangeable for the most part, except when subchromosomal aberrations, such as copy number variations, are included in CIN. Above the cellular level, the stability of tissue/organs and the immune system are also important. Even an individual's lifestyle can have an impact on cancer [6, 7]. For example, the contribution of stress-related psychological factors to cancer incidence and survival has gained attention [120, 121]. The survival of breast cancer patients increases 25–28 % in groups with family or social support as opposed to patients without support, while depression can increase mortality by 40 %. Note that here the psychological contribution is more significant than most genetic factors. This fact underscores the importance of the high level of constraint in genome-mediated cancer evolution [7]. It should be pointed out that these higher levels of stability closely relate to an individual's overall genomic instability. The rationale of emphasizing that CIN is more important than other cellular, tissue, or organismal levels of instability is that cancer is an evolutionary process where inheritance is the key component. Factors occurring above the genome level are not inherited while chromosomes are responsible for system inheritance. Irrespective of the influence of these higher levels, genome-level alterations must be involved in order for them to impact cancer evolution. On the other hand, compared with lower-level genetic and nongenetic alterations, chromosomes and the genome represent the highest level of genetic organization. According to the genome theory, even though lower-level instability can contribute to CIN, chromosomes play the central role in the organization of genetic systems. Determining whether or not other factors matter (genetic or nongenetic factors including epigenetic factors and environmental factors) in cancer evolution is mainly dependent on the emergence of a new genome system. The newly formed genomic package includes a unique gene and epigenetic profile with unique potential responses, as the genome defines the boundary of a system.

8 Conclusions and future perspectives

Clearly CIN is the driving force behind most cancer evolution and the genomic basis of poor prognosis. The research focus should shift to harnessing the behavior of unstable genomes through better understanding the patterns of evolution rather than continue to attempt to link diverse individual molecular mechanisms to CIN. Cancer is a stochastic evolutionary process and as the various cancer genome projects have shown, identifying "driver" mutations, the central tenet of the stepwise accumulation of gene mutations model, is of limited practical clinical value [6, 122, 123].

CIN does raise some issues that demand immediate attention. First, a standardized framework regarding CIN is needed. In order to develop this framework we must determine the following issues: What types of numerical and structural aberrations should be measured and how can the quantitative contributions of each be determined? Should we focus on NCCAs or CCAs? With regard to basic research in model systems during which time points should genomic dynamics be measured, immediately after the genetic/epigenetic manipulation or after longer periods of time, or should they be measured during multiple stages? Although genome-level organization is the most important genetic level to measure to determine the evolutionary potential of a tumor, how do we integrate other levels of genetic alteration?

Second, comparative studies between various genetic/epigenetic instabilities are needed within defined phases of somatic cell evolution. Recent reports demonstrate that genome-level alterations provide better predictability than gene mutation profiles [23, 124, 125]. It is thus necessary to use defined systems to quantitatively compare the contributions of genome alterations and determine evolutionary potential. For example, we are now actively comparing contributions of karyotypic and epigenetic profiles. An important area of future research is to

compare the contributions of subchromosomal level instability (such as copy number variation), structural CIN and numerical CIN relative to their tumorigenicity and response to drug treatment. In addition, the issue of how CCAs and NCCAs can be used together when scoring CIN needs to be addressed, as they represent stability and instability respectively, and the combinational effect is not easily determined.

Third, integration of information regarding the nature of CIN into diagnosis and treatment protocols is central to improving cancer care. Measuring CIN is not always straightforward, and standardized methods of doing so must be developed. New technologies also need to be developed. Currently, acquiring sufficient mitotic cells from tumors for SKY analysis is challenging and alternative methods such as interphase FISH need to be used [5, 126]. Recently we have measured the frequencies of NCCAs in peripheral lymphocytes to evaluate the contributions of overall instability in individual patients with different cancers and common diseases [72, 127] (Heng et al., unpublished data). In regards to cancer treatment, it has been suggested that CIN can destabilize aneuploidy increasing the efficiency of chemotherapy [128]. Targeting karyotype complexity and CIN in cancer cells has also been proposed [129]. This strategy requires caution however. As we have illustrated, when subjected to high levels of stress, the evolutionary phase can switch and unstable genomes will transition to genome chaos [20, 22, 60, 61, 130]. Genome chaos represents a new mechanism for the rapid development of drug resistance [70, 90, 91]. One form of genome chaos, chromothripsis, has recently been detected in multiple types of cancer [131]. Therefore, the existence of genome chaos is a real problem for targeting CIN. According to our experience, promoting genomic instability enhances cell death in the short term, but these already chaotic genomes can undergo further macroevolution promoting cancer survival in the long run. Furthermore, targeting CIN may negatively impact normal cells by inducing CIN in them. Similarly, use of epigenetic sensitizations used for promoting cell death requires caution. Successful treatment of cancer requires a new strategy of constraining cancer by applying evolutionary principles. For example, regulating the phase of evolution rather than directly hitting cancer cells hard with the intention of shrinking tumors and inducing rapid genome evolution is an attractive strategy. In light of the evolutionary principle of cancer, there are some obviously preferred treatment avenues: (1) lower treatment dosages that apply moderate pressure to constrain tumor growth and metastasis without accelerating evolution in cancer cells (not to induce or promote genome chaos, for example). (2) Maintaining or prolonging the stepwise phase of evolution without provoking a shift to the punctuated phase in order to avoid stimulating macroevolution. (3) Enhance higher level system constraints without directly targeting the cancer cells including improving immune function and maximizing nutrition status and psychological health. Diagnostic procedures and treatments should be developed to reduce stress on patients and stabilize CIN status by controlling introduced stresses [34]. The key to all of these potential strategies is to balance short term tumor responses with long term patient benefits. As we have pointed out, the ultimate goal of successful cancer treatment is to prolong and improve the quality of life of patients, rather than base success solely on the initial reduction of tumor size. There clearly is a gap between molecular targeting to maximally eliminate cancer cells and patients' overall benefit (longer survival and less suffering) [91, 132].

Last, despite the importance of serving as a common mechanism of cancer evolution, having CIN present does not necessarily mean an individual cancer will automatically progress. In other words, all cancers display CIN, but not all CIN leads to cancer progression which is dependent on multiple factors, each of which contributes a certain probability. For example, it is clear that smoking causes lung cancer in some cases, and approximately 80 % of smokers will not progress to cancer, though they may incur other diseases. Similarly though a specific mutation or allele may contribute to an increased cancer incidence, many individuals with that mutation will not acquire cancer even at an old age.

Given the fact that so many cells could acquire genetic/nongenetic errors as there is a large number of different stresses humans face every day, we need to appreciate that cancer evolution must overcome many obstacles to be successful [133–135]. Under experimental conditions, researchers can artificially speed up evolution and illustrate a so-called causation between a given molecular mechanism and cancer, but in reality with respect to nonlinear evolution, selected individual molecular mechanisms have limited predictability. In patients, multiple levels of systems including tissue/organ/immune systems, and time factors, which constantly alter the evolutionary selective landscapes, make it very difficult to predict whether given cells will "win the cancer evolution race." Clearly, more attention needs to be given to the effect of CIN on genome-based evolution, particularly over time periods (early, late cancer stages, and prior, during, and after medical interventions). The new genomic science called 4D-Genomics has come of age to address these issues [7].

Acknowledgements This review/synthesis is part of a series of studies entitled, "The mechanisms of somatic cell and organismal evolution." We would like to thank Gloria Heppner, Gary Stein, O.J. Miller, and Avraham Raz for their continuous support. This work was partially supported by grants from the DOD (GW093028), the National CFIDS

Foundation, the Nancy Taylor Foundation for Chronic Diseases, and SeeDNA Biotech Inc.

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