PROSPECT
Journal of Cellular Biochemistry 109:1072–1084 (2010)

Journal of Cellular Biochemistry

The Evolutionary Mechanism of Cancer

Henry H.Q. Heng,[1,2,3]* Joshua B. Stevens,[1] Steven W. Bremer,[1] Karen J. Ye,[4] Guo Liu,[1] and Christine J. Ye[5]

[1]*Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan*
[2]*Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan*
[3]*Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan*
[4]*SeeDNA Biotech, Inc., Windsor, Ontario, Canada*
[5]*Department of Internal Medicine, Wayne State University School of Medicine, Detroit, Michigan*

ABSTRACT

Identification of the general molecular mechanism of cancer is the Holy Grail of cancer research. Since cancer is believed to be caused by a sequential accumulation of cancer gene mutations, the identification, characterization, and targeting of common genetic alterations and their defined pathways have dominated the field for decades. Despite the impressive data accumulated from studies of gene mutations, epigenetic dysregulation, and pathway alterations, an overwhelming amount of diverse molecular information has offered limited understanding of the general mechanisms of cancer. To solve this paradox, the newly established genome theory is introduced here describing how somatic cells evolve within individual patients. The evolutionary mechanism of cancer is characterized using only three key components of somatic cell evolution that include increased system dynamics induced by stress, elevated genetic and epigenetic heterogeneity, and genome alteration mediated natural selection. Cancer progression represents a macro-evolutionary process where karyotype change or genome replacement plays the key dominant role. Furthermore, the recently identified relationship between the evolutionary mechanism and a large number of diverse individual molecular mechanisms is discussed. The total sum of all the individual molecular mechanisms is equal to the evolutionary mechanism of cancer. Individual molecular mechanisms including all the molecular mechanisms described to date are stochastically selected and unpredictable and are therefore clinically impractical. Recognizing the fundamental importance of the underlying basis of the evolutionary mechanism of cancer mandates the development of new strategies in cancer research. J. Cell. Biochem. 109: 1072–1084, 2010.

KEY WORDS: GENOME THEORY; CANCER EVOLUTIONARY MECHANISM; MACRO-EVOLUTION; GENOMIC INSTABILITY; NON-CLONAL CHROMOSOME ABERRATIONS (NCCAs)

Cancer research is at a crossroad. The generally accepted concept of tumorigenesis states that cancer is caused by a sequential mutation of oncogenes and tumor suppressor genes [Hahn and Weinberg, 2002; Vogelstein and Kinzler, 2004; Wood et al., 2007]. Now this gene-centric concept of cancer is seriously challenged by the success of cutting edge genomic technologies. Intending to solve the increasing confusion in the field due to the failure to identify the long expected patterns of genetic alterations universal to most cancers, the cancer genome sequencing project was launched [Collins and Barker, 2007] based on the assumption that cancer heterogeneity among patients is genetic ''noise'' and could be eliminated by validation using large patient samples. However, this costly approach is revealing even greater genetic heterogeneity [Greenman et al., 2007; Wood et al., 2007; Heng, 2007a; Ley et al., 2008; Parsons et al., 2008]. The vast majority of gene mutations are different among patients or even within the same tumor [Bielas et al., 2006]! With more cancer samples being sequenced, the list of mutated genes has increased extensively coupled with the identification of more diverse molecular mechanisms, not to mention that the epigenome project will soon provide even more levels of heterogeneity and complexity [Jones et al., 2008a; Heng et al., 2009]. The most challenging issue will not be linking gene mutations or epigenetic alterations into known pathways but will be predicting disease conditions and possible responses following medical intervention based on the high levels of diverse gene mutations or epigenetic alterations.

Increasing numbers of investigators are searching for alternative causes including cancer stem cells [Schatton et al., 2009], metabolic

Grant sponsor: Susan G. Komen Breast Cancer Foundation; Grant sponsor: SeeDNA Biotech, Inc..
*Correspondence to: Dr. Henry H.Q. Heng, PhD, Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48201. E-mail: hhqheng@gmail.com
Received 7 December 2009; Accepted 14 December 2009 • DOI 10.1002/jcb.22497 • © 2010 Wiley-Liss, Inc.
Published online 8 March 2010 in Wiley InterScience (www.interscience.wiley.com).

stress and errors [Jones and Thompson, 2009], endoplasmic reticulum (ER) stress [Moenner et al., 2007], oxidative damage [Martien and Abbadie, 2007], ubiquitylation [Miasari et al., 2008], aneuploidy [Duesberg et al., 2000, 2006; Shen et al., 2005], infection and inflammation [Greaves, 2006; Mantovani et al., 2008; Wu and Zhou, 2009], tumor/tissue interaction [Bissell et al., 2005; Nelson and Bissell, 2006], immunodeficiency [Klein and Klein, 2005], diet nutrients [Huang, 2002], and a large array of epigenetic effects [Baylin, 2005; Jaffe, 2005; Feinberg et al., 2006; Zaidi et al., 2007; Dolinoy and Jirtle, 2008; Esteller, 2008; Delcuve et al., 2009; Sharp, 2009]. These diverse approaches targeting different genetic/epigenetic and cellular targets represent the same attempt to find common causative patterns. Will one of these approaches finally provide the long sought after magic bullet? The answer is no. Many of these popular approaches come and go, representing another wave of fashionable research [Harris, 2005; Heng, 2007a]. This situation is analogous to the classic story of the blind men and an elephant. No single molecular mechanism represents a universal pathway for cancer, what then is the correct mechanism of cancer?

Last year, three important meetings were organized by the US National Cancer Institute. These think tank meetings brought together top scientists from physics, physical chemistry, engineering, mathematics, cancer biology, and clinical oncology to collaboratively identify the most promising research questions and strategies in cancer research. Not surprisingly, the physics of carcinogenesis, cancer evolution, system complexity, and information science were identified as top priorities. According to the NCI leadership, these priorities are the next logical step in moving the field forward (http://www.cancer.gov/ncicancerbulletin/NCI_Cancer_Bulletin_110408/page4). An understanding of the evolution of complex cancer systems will not only unify these key priorities (as both the physical characterization and information issues are important components of somatic cell evolution) but also provide the urgently needed conceptual framework and methodologies for cancer research. Without it, we are left with only stacks of contradicting case reports. The key challenge, therefore, is to illustrate the evolutionary mechanism of cancer and to apply this principle to guide our research as well as clinical applications.

In addition to the cancer genome sequencing projects, an array of cutting edge genomic and proteomic technologies has been applied to cancer research, including gene duplication studies [Feuk et al., 2006], gene global expression and genomic analysis [Liu, 2004; Fan et al., 2006], single DNA molecule detection of mutations [Bielas et al., 2006], large-scale genome methylation studies, and individual cell karyotypes among cell populations both in vitro and in vivo [Heng et al., 2006a,b,c; Bartos et al., 2007; Ye et al., 2007; Ke et al., 2009]. Collectively, the key information derived from these diverse studies is the extremely high degree of genome system complexity reflected as multiple levels of genetic and non-genetic (including epigenetic) heterogeneity [Brock et al., 2009; Heng et al., 2009].

Based on these new findings, overwhelming yet previously ignored evidence has been recently re-synthesized and formed the genome theory of somatic cell and organismal evolution [Ye et al., 2007; Heng, 2008a, 2009]. This new concept does not focus on the identification of common patterns with specific molecular targets (as there are no such commonly shared highly penetrant targets) but focuses on system dynamics and how genome evolution creates new bio-systems. In contrast to the gene-centric approaches, the genome theory maintains the importance of genome level alterations and selection that serves as a driving force for somatic cell evolution. In support of this new concept, the evolutionary mechanism of cancer has recently been established and linked to the total collection of large numbers of diverse individual molecular mechanisms [Ye et al., 2009]. The realization that the evolutionary mechanism is more significant than any individual molecular mechanism and that entire fields have been working on individual molecular mechanisms places emphasis on the need for development of new strategies to deal with the nature of complex systems and how they affect cancer evolution. If almost every factor can contribute to cancer evolution by producing system stress, then any given individual factor would have limited predictive power. Therefore, focus should be placed on the evolutionary patterns of a system's response to stress rather than on any specific stress to a system as often there are many stress factors to be considered that could contribute to the causal basis of cancer.

In this prospect, we will briefly review the concept of cancer evolution, and a few key components of somatic cell evolution and how it drives cancer progression through genome alteration mediated macro-evolution. The rationale and research that led to this discovery of the evolutionary mechanism of cancer and its relationship to all the other molecular mechanisms are described. Finally, the implications to basic research and clinical studies of this concept as well as some new directions in cancer research will be discussed.

CANCER FORMATION IS AN EVOLUTIONARY PROCESS

All necessary and sufficient conditions for natural selection can be found in the process of cancer formation [Crespi and Summers, 2005; Merlo et al., 2006; Heng et al., 2006a,b,c, Heng, 2007a; Ye et al., 2007; Gatenby, 2009]. There must be heritable variation (either genetic or epigenetic) in the population, and the variants must display differential fitness (affecting either survival or reproduction of cell populations). The fact that cancer cells compete with normal cells for resources (nutrition and space) and the observed clonal expansion from both in vitro models and in vivo tumor samples strongly supports the conclusion that cancer initiation–progression and acquisition of drug resistance represent typical evolutionary processes.

The initial idea that linked evolution to tumors can be traced back to over a century ago, when Boveri connected the dots between abnormal chromosomes, inheritance among parental–daughter cells, and cancer [Manchester, 1995]. In addition to the attempt to develop the somatic evolutionary theory of cancer through mathematical modeling (as a process of sequential accumulation of somatic mutations) [Armtage and Doll, 1954], additional evidence has come from chromosomal studies that led to the hypothesis that cancer evolves through a sequence of chromosome aberrations and the selection process [Yosida, 1966; de Grouchy et al., 1966]. In addition to the contributions of Knudson [1971] and Cairns [1975],

Nowell's [1976] evolutionary view of cancer received the most attention. By emphasizing the importance of genetic instability and natural selection, Nowell's idea initiated a series of evolutionary studies that identified clonal expansion within many types of tumors [Brentnall et al., 1994; Maley et al., 2006].

It is interesting to mention that the introduction of the cancer evolution concept at that time and even now have failed to revolutionize the field of cancer research. Today, most molecular geneticists perceive cancer evolution as just a theoretical concept which seems to have little relevance to their molecular characterization of gene mutations and cancer-specific pathways. It has been reasoned that even if cancer progression is an evolutionary process, such a process is still defined by common cancer gene mutations. It seems that according to this thinking somatic cell evolution does not matter, as long as we can identify the key cancer genes. For those who are interested in cancer evolutionary studies, many have been focused on clonal expansion, as recurrent types of changes can be traced to illustrate the evolutionary path. It has been thought that clonal expansion is the basis for accumulating mutations based on the gene-centric concept of cancer evolution. In contrast, the importance of high levels of heterogeneity in somatic evolution has been more or less ignored. For example, in the formation of solid tumors, there is an involvement of multiple cycles of clonal and non-clonal expansion [Heng et al., 2006a,b,c; Ye et al., 2007]. Even during the typical clonal expansion phase, there are significant levels of heterogeneity within a cell population; however, most are underdetected when mixed populations of cells are used for molecular analysis. Furthermore, most of the somatic cell evolution studies have been mainly limited to gene level analysis, which overlooks the main feature of genome alteration mediated macro-evolution that drives cancer evolution [Cahill et al., 1999; Tsao et al., 2000; Merlo et al., 2006; Nowak et al., 2006; Beerenwinkel et al., 2007; Heng, 2009].

UNIQUE FEATURES OF SOMATIC CELL EVOLUTION

It has been puzzling why somatic cell evolution occurs so rapidly (a few decades in most cancer evolution cases) when compared to organismal evolution where millions of years may be involved. This time-scale difference has increased doubts for some researchers who consider cancer evolution and organismal evolution to be fundamentally different. A key breakthrough came from the study of karyotypic evolution during the immortalization process using an in vitro cell culture model [Heng et al., 2004a, 2006a,b,c]. Multicolor spectral karyotyping [Heng et al., 2001, 2003; Ye et al., 2001, 2006] was used and karyotypic evolution was analyzed both in individual cells and cell populations following various stages of cellular immortalization. This study revealed the following interesting findings:

(a) Genome-based macro-evolution (reflected as the formation of new karyotypes) serves as the driving force for immortalization.
(b) There are two phases of karyotypic evolution. One is the discontinuous phase within which the karyotypes are not traceable between different passages of culture or even within the same passage; the other phase is the classic stepwise continuous phase within which karyotypes can easily be traced for hundreds of passages, and the majority of cells share similar karyotypes. Importantly, these two phases represent punctuated (or macro-evolution) and Darwinian (or micro) evolution, respectively, and these two distinctive phases are part of the same evolutionary process (Fig. 1).
(c) The two phases were co-mapped with system stability. System stability was measured by the level of stochastic genome alterations. When genome systems are unstable evidenced by high frequencies of non-clonal chromosome aberrations (NCCAs), then evolution enters into the punctuated phase. When genome systems are relatively stable, as illustrated by dominant clonal chromosome aberrations (CCAs) and low frequencies of NCCAs, then evolution enters the Darwinian stepwise phase.
(d) In the unstable phase, genome-level heterogeneity is extremely high as every cell is drastically different at the genome level. This important observation, coupled with the fact that karyotypes define a given genome system, provide the genetic underpinning of the high degree of heterogeneity that is universally detected in cancers [Foulds, 1954; Dexter et al., 1978; Heppner, 1984; Heppner and Miller, 1998; Aubele et al., 1999; Gonzalez-Garcia et al., 2002; Bartos et al., 2007; Bayani et al., 2007; Ye et al., 2007; Jones et al., 2008b].
(e) By repeating the same experiments, or analyzing the parallel clones derived from the same initial cell population, the immortalized cells display unique distinctive karyotypes, demonstrating the stochastic nature of karyotypic evolution during cellular immortalization.

Additional follow-up experiments demonstrated that genome-based macro-evolution can be detected in most of the major transition steps in cancer including immortalization, transformation, metastasis, and drug resistance [Heng et al., 2008]. The common pattern of genome system replacement during all these major transitions also supports the concept that karyotypes define bio-systems; and that karyotypic evolution is the key event in cancer evolution.

Together, these analyses challenge the traditional evolutionary concepts regarding somatic cell and organismal evolution. In particular, the discovery of the surprising relationship between macro- and micro-evolution calls upon the need to re-evaluate the contributions of gene mutations and chromosome aberrations during cancer progression. For example, the importance of high levels of "genetic noise" observable as karyotypic heterogeneity in cancer evolution need to be determined as well as deciding the meaning of specific gene mutations or pathways when stochastic karyotypic alterations occur. It is also important to determine the key differences between macro- and micro-evolution, and key features between somatic cell and organismal evolution.

The evolutionary dynamic patterns of prokaryotes and eukaryotes have been compared with the two phases and their patterns of somatic cell evolution. Interestingly, the punctuated phase mimics prokaryotic evolution, while the Darwinian phase mimics

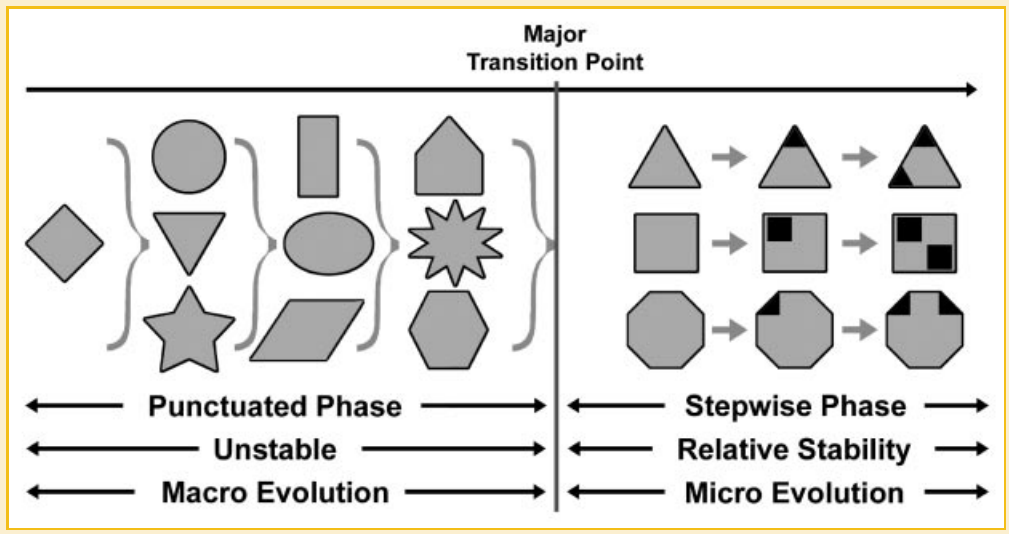

Fig. 1. A diagram showing the two phases of karyotypic evolution observed from major transition processes of cancer progression (e.g., immortalization, metastasis, drug resistance). The different shapes represent different karyotypes (genome systems). Within the punctuated dynamic phase, at the beginning of treatment, all cells display relatively stable karyotypes, during progression or treatment, most of the karyotypes alter under stress, and there is high level of non-clonal chromosome aberrations (NCCAs) detected indicating an unstable stage. There are different dominant karyotypes detected (clonal chromosome aberrations or CCAs), but none of them survive for long. They are constantly replaced by new genomes through genome shattering. Within the Darwinian stepwise phase, in contrast, dominant karyotypes (CCAs) emerged and last a much longer time. The three examples illustrated in this phase represent stochastically formed CCAs derived from the same cell population. According to the genome theory macro-evolution occurs during this punctuated phase where highly dynamic genome system replacement dominates; while microevolution occurs during the stepwise or Darwinian phase where dominant karyotypes exist for long periods of time (illustrated by the same shapes but with minor modifications over the time).

eukaryotic evolution where karyotypic relationships can be used to build an evolutionary tree. This comparison has surprisingly revealed the relationship between sexual reproduction and system stability. In brief, we have linked the punctuated phase to the dynamics of asexual species and the Darwinian phase to sexual reproduction phase (by hypothesizing that the main function of sex is to maintain the system identity by filtering out altered genomes). The significance of this work is obvious. First, it provides a convincing explanation for the main function of sex as limiting rather than increasing genetic diversity [Heng, 2007b; Glansdorff et al., 2009; Gorelick and Carpinone, 2009; Wilkins and Holliday, 2009]; second, it states that the evolutionary patterns of prokaryotes and eukaryotes (with sexual reproduction) are fundamentally different; third, it points out the conflict between the gene and genome levels. Specifically, the main function of the genome is to reduce diversity at the genome level to maintain system identity, and at the gene level the recombination mechanism can promote diversity to provide additional features within a given system; and lastly, it illustrates a key difference between somatic cell evolution and organismal evolution. Somatic cell evolution displays observable patterns of both prokaryotic (punctuated macro-evolution) and eukaryotic (stepwise/Darwinian or micro) evolution. Despite the fact that punctuated evolution was introduced to describe phenotypic changes in multicellular organisms at a time scale much greater than cellular evolution [Gould and Eldredge, 1993], the concept fits well with prokaryotic and somatic cell evolution in terms of the pattern of genomic macro-evolution. Furthermore, due to the absence of a sexual filter that can constantly preserve the genome system, somatic cell evolution is much more quickly mediated by the accumulation of drastic genome level alterations. Now, it is understood that for organismal evolution, if we study the micro-evolution within a species, there are no karyotypic changes no matter how many generations occur. In cancer evolution, however, the key is the replacement of the karyotypes, and therefore, the main driving process is genome alteration mediated macro-evolution.

Another difference between somatic cell and organismal evolution is that, in terms of macro-evolution, each tumor is a successful independent run of evolution, thus many cases together illustrate multiple runs of evolution. In contrast, a eukaryote's precedent historical evolution is only one possible run of evolution. For any given single run of evolution, it is much easier to trace the evolutionary tree as there is much less stochasticity when compared to multiple runs of evolution.

THE UNDERSTANDING OF THE EVOLUTIONARY MECHANISM OF CANCER AND ITS RELATIONSHIP WITH OTHER MOLECULAR MECHANISMS

Based on these newly discovered features of somatic cell evolution, we have attempted to establish the evolutionary mechanism of cancer. To achieve this goal, we have carried out three types of experiments and synthesis in the past 10 years.

First, we have illustrated that, various factors, genetic and non-genetic alike can cause the genome system to become unstable as measured by the elevated level of NCCAs. The factors examined include gene mutations related to genetic instability (ATM, p53); expression of oncoproteins, carcinogenic treatment, ER stress, the aging process, nutrition status, and cell culture conditions

[Heng et al., 2004b, 2006a,b,c, unpublished work; Shen et al., 2005]. Overwhelming evidence can be found in the literature linking the unstable genome to infections, wound healing, and the micro-environment [Radisky et al., 2005; Krizhanovsky et al., 2008; Heng et al., 2009; Lacoste et al., 2009]. Most significant, we have recently linked most of the factors that induce higher levels of NCCAs to system stress. In other words, no matter what type of treatment or molecular changes we introduce into a system, they all function as stress to the involved system. Under stress, regardless of its type or where it comes from, the general consequences of stress are to increase the level of system dynamics, especially when the stress is high or the system being examined is unstable [Heng et al., 2010].

Second, as we have demonstrated, when genome systems are unstable as induced either by various treatment or caused by the system itself (such as telomeric shorting), the cell population heterogeneity often increases. Under selection pressure, the level of NCCAs will decrease in many populations some however under the same culture conditions, will remain in the unstable phase for a much longer time. When the degree of genome instability is moderate, new CCAs often will not form within a short period of time. By changing the selection conditions or by longer periods of selection even under the same conditions, there will be more opportunities for new CCAs to be established [Ye et al., 2007]. It should be pointed out that even under similar selection conditions, the formation of CCAs seems to be a stochastic process so that different CCAs will be formed from different groups. The elevated dynamics level of a system under stress often will not have a fixed response.

Third, we have demonstrated that, genome-based cell population heterogeneity is linked to tumorigenicity [Ye et al., 2009]. Using six well-characterized in vitro tumor progression models representing various types of cancer including human breast and prostate cancers as well as mouse ovarian cancer, we asked the following question: outside of models representing different types of cancer or different cases of the same type of cancer, what is the common feature if we consider them as independent systems using the somatic cell evolutionary principle? When compared with their molecular mechanisms, different cases of cancer are clearly very different, ranging from micro-satellite instability, to downregulation of E-cadherin and expression of A2 and E2Fl. Even in the four breast cancer models that were derived from the same MCF10 system, distinctive molecular mechanisms were involved including centrosome amplification, activation of cdD1 and Bcl-2, increased expression of PCNA and gadd45, and altered stromal–epithelial interaction. When comparing the end products of evolution for all sublines of these model systems, however, they can easily be classified as two groups: the group with high tumorigenicity demonstrated by both in vitro and in vivo assays and the group with low tumorigenicity. Clearly, the end products of evolution are much less diverse compared with the pathways. What about the cancer evolutionary process for the sublines of these various models? When comparing the alterations at the genome level with spectral karyotyping, a clear picture has emerged. In all sublines displaying high tumorigenicity, regardless of which molecular mechanisms were detected, they all share the high levels of genome heterogeneity, illustrated by the high frequencies of NCCAs! In contrast, for all sublines with low tumorigenicity, they displayed distinctly lower frequencies of NCCAs. The common link is genome system heterogeneity! According to the evolutionary principle, it makes perfect sense that system heterogeneity, especially at the genome level serves as key material for cancer evolution.

The evolutionary mechanism of cancer can be described in three components or steps (Fig. 2): (1) stress-induced genome system instability (the diverse causes of cancer); (2) this instability produces genetic and epigenetic heterogeneity (A diverse population is essential for evolution); (3) somatic cell evolution, mainly macro-evolution, is based on a series of genome system replacements which breaks the multiple system constraints (such as the tumor suppressor function of genome integrity, tissue architecture, and immunesystem safeguards).

Interestingly, there is a simple relationship between the evolutionary mechanism and diverse molecular mechanisms, and that is,

$$\text{Evolutionary mechanism} = \sum \text{all individual molecular mechanisms}$$

The evolutionary mechanism of cancer defines how system stress drives genome replacement (macro-evolution) leading to cancer, where the individual molecular mechanisms are linked to different genetic and epigenetic and non-genetic alterations. These alterations either can serve as a stress to the genome system (an initial condition that is essential for evolution) or provide new interaction between genetic elements participating in alternative pathways essential for somatic cell evolution. Based on our analysis, all factors, genetic or non-genetic alike, internal or external alike, as long as they function as a detectable stress to a given system, can all contribute to cancer evolution (either through micro- or macro-evolution). On the other hand, it is not easy for cancer evolution to be successful as there are multiple levels of system homeostasis existing. The vast majority of cells will not be able to attain the threshold of cancer without forming new genomes [Hanahan and Weinberg, 2000; Heng et al., 2008], despite the fact that many molecular pathways and combinational pathways have been linked to one of the hallmark phenotypes. The most effective way to drastically increase the probability of successfully progressing to cancer is through genome-level alterations (genome system replacement). When each individual tumor forms, if considered as an end product of evolution, can be characterized by known pathways. However, it is extremely difficult to predict which pathways will become dominant prior to tumor formation. In addition, current molecular analysis that is based on mixed cell populations can only profile the ''average molecular pathways'' which can be very misleading. According to our recent studies, most tumors are composed of cells that are not karyotypically clonal indicating that cancer was attained through different pathways. This is based on the concept that different karyotypes define different genome systems [Heng et al., 2008; Heng, 2008a], and that the same pathways can change function within different systems. Therefore, characterizing a pathway is less meaningful to understand the general mechanism of cancer and to ultimately understand how

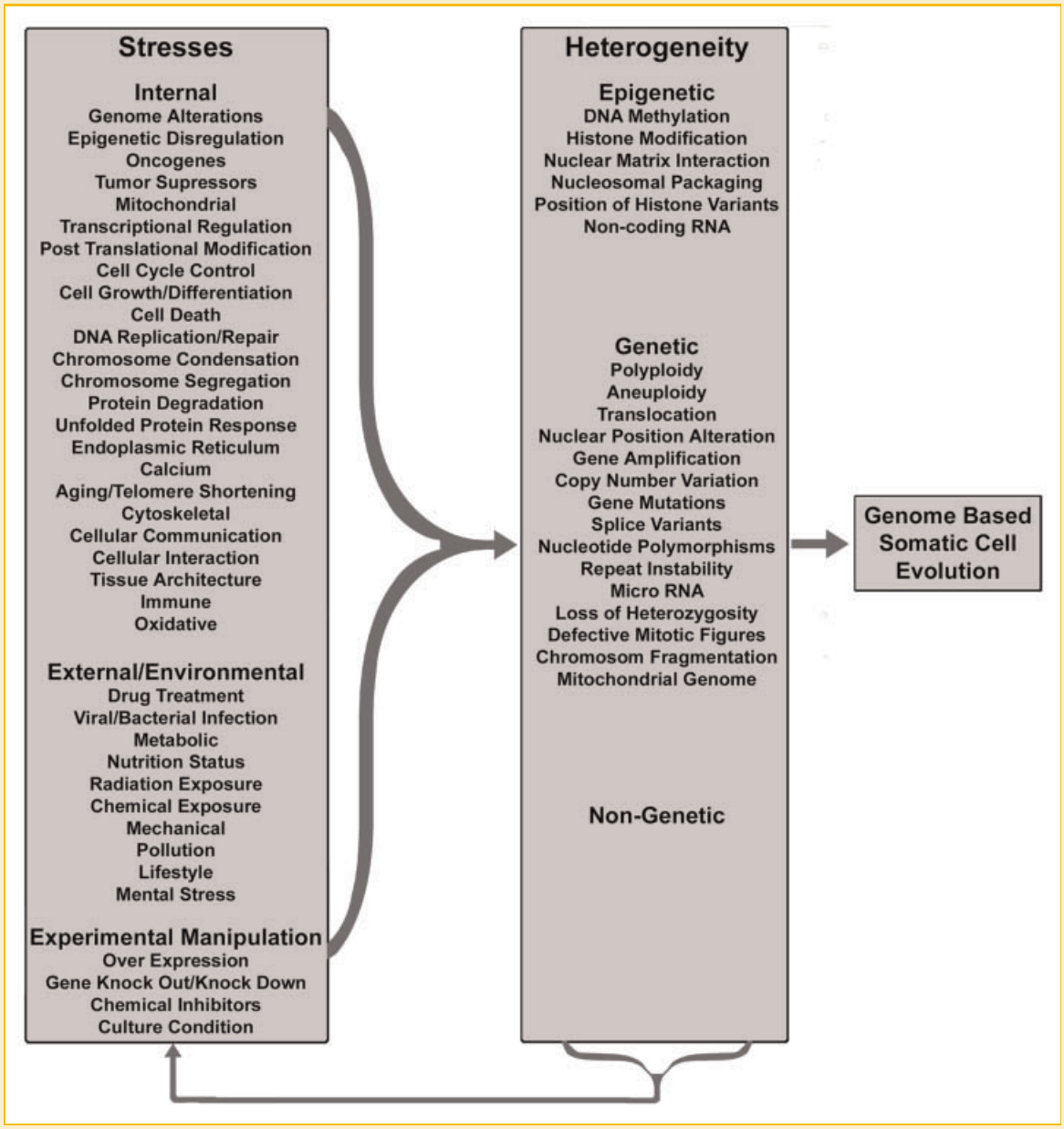

Fig. 2. A diagram that shows the interactive relationship between stress, system heterogeneity, and genome-based somatic cell evolution. For a large number of individual molecular mechanisms, their alterations will generate system stress, which increases the genome system dynamics and is reflected as increased genetic and epigenetic (also non-genetic) heterogeneity which is acted upon by natural selection. Additional complexity is a result of system instability (caused by any specific molecular mechanism), stochastically generating system heterogeneity. In return, the newly formed system heterogeneity can create new stress in the system at higher levels. A few examples of molecular mechanisms are listed to illustrate this point. It should be noted that, for each category listed, there is a great deal of variation. For example, the number of gene mutations can reach to billions per tumor and the number of histone modifications could have millions of possibilities. The combination of all these probabilities is potentially infinite. However, most stress-induced system dynamics will not lead to genome system replacement in vivo, and most of the newly formed genomes will not be able to become the dominate cell population due to constraints at multiple levels. Therefore, cancer is the result of evolutionary probability.

cancer can be clinically managed. Interestingly, there is very little analysis on most of the clones which do not reach the cancer stage. We predict that, most of them will similarly display a domination of some known molecular pathway. The key message here is: the most significant alterations are the ones that affect the phenotypic selection and are directly related to the genome context. Less important are the individual genes or pathways due to the unpredictable nature of pathway replacement during genome evolution, even though individual pathways can contribute to and often can be detected at a given stage of cancer progression by ''average detection methods'' (e.g., monitoring the specific proteins with Western blot analysis by averaging the profile of a mixed cell population, which could be very different from individual cells that provide the heterogeneity for evolution).

With that said, despite the fact that no individual molecular mechanism can serve as a general mechanism in the majority of cancer cases, from a purely academic point of view, it is still interesting to characterize individual mechanisms and determine each mechanism's level of contribution to the process as a whole. Given the fact that there have not been any dominant gene mutations identified, and that cancer evolution can proceed through many different mechanisms, a much more productive research goal would be to identify mechanisms related to genome dynamics and macro-evolution. Specifically this can be achieved by using genome level diversity as a new biomarker to measure, in the clinic setting, system instability and determine the likely potential for cancer formation and treatment responses.

GENOME THEORY OF CANCER EVOLUTION

As the total number of individual molecular mechanisms is very large (and the combination of molecular mechanisms can reach an unmanageable number), it is important to move our research from the characterization of individual molecular mechanisms to the understanding of the overall system behavior during cancer evolution. To do so, we need to switch from gene-centric thinking to genome-centric thinking. To achieve such an important transition, we have recently introduced the genome theory of cancer and organismal evolution [Ye et al., 2007; Heng, 2009; Heng et al., 2009]. The following concepts are directly related to somatic cell evolution:

(a) The genome represents the highest level of genetic organization. The relationship between the genome and genes can simply be referred to as the relationship between the "whole" system and the "parts." The whole is not equal to the sum of the parts and so the genome is not equal to the sum of all the genes. The information at the genome and gene levels is very different and the properties of the genome cannot simply be determined from the genes. The conflicting relationship between DNA/genes and chromosomes/genome has been illustrated by experimental approaches. When identical human DNA segments are inserted into the mouse genome by transgenic approaches, newly formed meiotic chromatin loops are different in loop size depending on the location of insertions along the chromosomes (close to telomeres or in the middle of chromosomes, for example) [Heng et al., 1996], demonstrating that the structure of inserted DNA is constrained by chromosomal position. When 12 copies of human protamine genes were introduced into a mouse genome, only one copy was expressed at a given time for multiple copy tandemly arrayed transgenes. This is likely related to the association of the transcriptional machinery and nuclear matrix and illustrates that the chromatin loop domain constrains gene function [Heng et al., 2004b]. It is also recently discovered that there is a drastically conflicting relationship between genes and the genome when discussing the function of sexual reproduction [Heng, 2007b]. The main function of sex at the genome level is to reduce the diversity by eliminating drastically altered genomes, while at the gene level is to increase the diversity by genetic recombination. By "filtering" out altered genomes, the identity of the same genome system (the identity of species) is preserved. The recombination of different genes can be tolerated by the genome as they only modify certain features of the system but do not replace the system itself [Heng, 2009].

(b) The genome context, not individual genes or pathways, defines a given biological system. The genome context refers to all genes and regulation elements (including other types of genomic DNA) of a given system and their genomic topology within nuclei. This is different from the traditional molecular biological viewpoint that ignores the physical constraints of the position of and distribution of genes which defines their potential interactive relationships. The genome theory states that the interactive relationships represent the blue print of life. To illustrate this point, we have introduced the "shattering genome model" to hypothesize how the self-organization principle can organize a network according to the genomic geography among all genes [Heng, 2009]. For a given species (or system), the number of genes (parts) is sufficient for its biological functions. The important feature is how the genes are organized and interact. We hypothesize that the chromosomal composition and position of chromatin domains in the nucleus (a feature of karyotypes) provides the self-organization matrix to form the genetic network structure. Thus, the genes of a given karyotype form a specific interactive relationship and their functions are defined by the genome system.

(c) The genome is the principle platform of somatic cell selection. Particularly during macro-evolution when new karyotypes are produced. Macro- and micro-evolution represent different levels of changes. Macro-evolution is not simply achieved by the accumulation of micro-evolution. Macro-evolution creates new genomes and micro-evolution modifies the existing genome. In cancer progression, to achieve the cancer transition events (immortalization, transformation, metastasis, and drug resistance), macro-evolution is required. For gene systems to evolve, selection acts upon the entire genome rather than the individual parts (genes), thus the substantial majority of alterations at the gene level will not be simply subjected to positive or negative selection.

(d) The genome package determines the pattern of epigenetic changes and potential response to the environment. Among the multiple levels of genetic and epigenetic relationship, each level can influence the other, but the impact of the genome level is more dominant. The function of a specific gene or epigenetic level of change might be very different when the genome system alters. Genome-level alterations, particularly when drastic, lead to macro-evolution. Epigenetic and gene mutations often lead to micro-evolution.

(e) High levels of system heterogeneity are not "noise" but a key feature of system dynamics essential for system adaptation [Ye et al., 2007; Heng, 2008b; Heng et al., 2009]. It is important to note that there are high levels of stochastic alterations at the genetic and epigenetic levels and the regulation of many of the pathways are less precise than we would have hoped. The

heterogeneity issue is especially true of cancer formation, as some tumors occur through cell populations with highly diverse genomes.

(f) Somatic cell evolution is much more drastic than organismal evolution. Even though the patterns of somatic cell evolution are similar to prokaryotic evolution, the combinational change at the chromosome level in somatic cell evolution is more drastic and happens more quickly. Sexually reproducing eukaryotes have sexual filters that constantly eliminate altered genomes [Heng, 2007b], and evolution is much slower in this case than somatic cell evolution.

(g) In somatic cell evolution, the pattern of evolutionary dynamics during (either the stepwise or discontinuous phases) is determined by system instability. Both internal and induced instability can change the pattern of evolution.

(h) The evolutionary mechanism of somatic cell evolution is equal to the total collection of all the individual molecular mechanisms.

(i) To analyze the evolutionary potential, it is more effective to measure genome system heterogeneity (or population diversity) at the genome or cell levels rather than at the lower levels of gene or epigenetic alterations [Heng et al., 2009]. The diversity of karyotypes has recently been linked to tumorigenicity [Ye et al., 2009].

Clearly, the focus of the genome theory is distinctively different from the gene theory that considers genes to be the basic unit of genetic information as well as a key element in evolutionary selection. According to the genome theory, most genetic information cannot be defined at the gene level as the function of an individual gene is genome context dependent. The evolutionary mechanism of cancer is also a logical implication of the genome theory that focuses on the genome-mediated phenotypic selection rather than individual molecular pathways. Of course, the genome theory of somatic cell evolution is in its infancy. Increasing knowledge and additional principles will be included with the maturation of this theory.

IMPACT AND IMPLICATIONS

Even though it now seems obvious, it has taken decades to establish such a simple relationship between the evolutionary mechanism of cancer and diverse molecular mechanisms. Clearly, the establishment of this relationship is of great importance. First, it ultimately challenges the traditional concepts and approaches of current cancer research that have narrowly focused on individual molecular mechanisms. Historically, this seemed very promising when only a handful of common mutations were expected to cause cancer. Nowadays, reviewing any major cancer research journal, the vast majority of publications are using similar methods and even nearly identical presentations to characterize different gene mutations or pathways. The popular ''pattern'' of research articles is the ''discovery'' of one specific or a number of genes that are involved in cancer formation in experimental settings. Following a number of in vitro tumorigenicity assays it was found that either overexpression or reduced expression can generate tumors in vivo in immunodeficient mice (the research evidence). The clinical connection to these genetic aberrations has been claimed to be found in some patient samples (the clinical evidence). Finally, targeting specific therapy works well in vitro and in animal models which seems to hold promise for clinical studies. Each of these specific stories on individual causes is very promising, and there are so many of them. Yet, the big picture in cancer research now is rather confusing, as most of these stories seem to come and go despite people's efforts to find the next important story on what causes cancer. Knowing what we know today, continuing to focus on individual molecular mechanisms is not justified, particularly when the penetration of any single molecular mechanism is low among patient populations.

The realization that cancer occurs through an evolutionary mechanism explains why most of the published gene mutations/epigenetic alterations or experimental manipulations seem to cause cancer under experimental settings, and these characterized genetic mutations are detectable in patients, yet, there is no common pattern of gene mutation detected in patient populations in most cancers. While each individual molecular mechanism can explain some cases, there are so many cases with drastically different molecular mechanisms, it is a challenge to identify the clinically useful common pattern as we had hoped. In a sense, many of the established molecular mechanisms are correct in that they can cause cancer and are thus important, but they are less important in the search to control cancer as they only represent a small portion of the total potential causes of cancer. In addition, the individual molecular mechanisms can easily switch with each other during cancer progression and in particular during medical intervention. According to the organization principle of the genome, in a given system, there are many possible pathways based on the same gene set. When the dynamic levels of a system are changed, the pathways can be altered. Since cancer cell populations display extremely high levels of heterogeneity, such pathway alterations can be elevated within and especially among different genomes.

By understanding the evolutionary mechanism of cancer, other mysteries can be better explained. For example, only a certain portion of patients are affected by cancer even when placed under similar stress, such as smoking. For example, only 20% of heavy cigarette smokers develop lung cancer by 75 years of age in the absence of death by other causes. It is an issue involving genetics/stress and chance, based on evolutionary probability. One needs a ''perfect storm'' to develop cancer. Genetic and stress factors certainly can increase the probability of cancer, but it is not a sure thing in any individual.

At first glance, by understanding the mechanism of cancer evolution, it seems to offer less specific strategies to directly fight cancer. However, the new way of thinking is essential for the entire field to establish the right conceptual framework and to identify correct targets and to re-configure the effort and resources of cancer research. This paradigm shift will change the way we study and treat cancer by providing the fundamental concepts of the cancer process leading to the emergence of the correct path of the management of this terrible disease.

An important effect following the establishment of the evolutionary mechanism of cancer will be to promote cancer prevention.

Since stress and its mediated genome-level heterogeneity are keys to triggering cancer evolution, prevention should focus on how to reduce system stress and how to slow down the processes that increase genome-level heterogeneity. This can also be applied to drug treatments as well as to earlier diagnosis. For most drug therapies, the most common approach is to target the death of cancer cells. However, this introduces stress and that often can increase the degree of heterogeneity of a cancer cell population in the long run despite short-term induction of cancer cell death. The end result will be to speedup the process of drug resistance through cancer evolution [Heng et al., 2009]. Normal cells will be at a survival disadvantage as they are much less dynamic than cancer cells, particularly as many of the check point mechanisms within normal cells will not allow them to be drastically changed. As for early diagnosis, one also needs to consider the potential negative impact (only the benefits are typically discussed in the field), as many of the earlier lesions will not progress into clinically defined cancer. It is possible that the introduced stresses can in fact promote some of them to form resistant cancer. This important question needs to be carefully examined based on the evolutionary concept.

Another important implication of the evolutionary concept of cancer is to re-examine some of the long accepted beliefs which have guided our research efforts. For example, we have believed that knowledge of physiological (or developmental) conditions is directly transferable to pathological conditions, and vice versa. However, based on the genome theory, it is possible that the developmental or pathological processes are not the same involving different levels of system alterations. The developmental process is a well-controlled process where each aspect can be linked to a specific gene function for a given genome, while pathological changes such as cancer progression is a stochastic process involving genome replacement. As we have illustrated, genome replacement is fundamentally different from gene mutation [Heng, 2009; Heng et al., 2009]. Only genome replacement involves different genome systems. When pathological changes involve chromosome alterations, the information is no longer at the gene level. Therefore, there seems to be a knowledge gap that exists between gene-dominated physiological or developmental changes and genome-dominated pathological changes that might prevent us from simply transferring information. Our recent studies have further illustrated this point by linking various molecular targeting events to system stress. Most significant, one can control the specificity of molecular targeting (during the initial experimental phases), but as soon as the system becomes unstable, the outcome of the system is less predictable [Heng et al., submitted]. Similarly, this knowledge gap applies to the genome versus gene and specific case studies versus population prediction in biology.

The above-discovered knowledge gap brings into question one of the long held beliefs in the field that even though there may be many individual molecular mechanisms, if they are all known then we will be able to finally control cancer. Knowing all the individual mechanisms (even if this were possible) will result in very few clinical applications. First, it would be very challenging to list all of the combinations of mutation profiles and epigenetic changes, as each tumor hosts over a billion individual mutations. Then there is an issue of the knowledge gap. Yes, we probably can develop a map of pathways after many decades' of hard work (this map will be highly dynamic and not easily interpreted); however, knowing the map and knowing how the pathways can switch between each other (within and among different genome systems) means completely different things, not to mention the fact that the same pathway can have drastically different functions within different genomes. Obviously, we need to study the evolutionary mechanism of cancer by studying three key components (stress, genome heterogeneity, and natural selection). Despite not knowing many of the details at this stage, the hope lies with the approach to control cancer by influencing the patterns of system evolution.

In addition to shedding new light on cancer research, the evolutionary mechanism of somatic cell evolution also provides valuable understanding of organismal evolution. Due to the difficulty of examining long-term evolutionary processes in nature, as it is particularly difficult to have a time window long enough to observe macro-evolutionary events, many of the important issues in evolutionary theory are untested. The somatic cell evolution model can now be used for this purpose, as different systems share common patterns of system dynamics and patterns of evolution.

CONCLUSION

The concept of cancer evolution is not new to cancer researchers [Cairns, 1975; Nowell, 1976; Crespi and Summers, 2005; Merlo et al., 2006; Nowak et al., 2006; Heng et al., 2006a,b,c; Heng, 2007a, 2009; http://en.wikipedia.org/wiki/Somatic_evolution_in_cancer]. However, a number of key principles and their implications in somatic cell evolution have not been understood until now with the establishment of the genome theory [Heng, 2009; Heng et al., 2009]. To convince readers that the evolutionary concept is under appreciated in the cancer field, we would like to ask some simple questions: If it is known that cancer is an evolutionary process, and the key feature of evolution is its stochastic nature, then why are we still trying so very hard to identify universal patterns of genetic alteration in cancer? If somatic cell evolution is driven by genome alteration mediated macro-evolution, why do we still focus on the gene and pathway studies that are more relevant to micro-evolution? If the evolutionary mechanism of cancer is equal to the combination of all individual molecular mechanisms, and there are so many different types of molecular mechanisms, how useful is it to try to establish individual molecular mechanisms? The chilling fact is, we do not know or are unwilling to believe the important implications of evolution on our cancer research.

To move the field forward, we have to apply the evolutionary principle not only to shake up the conceptual framework of cancer research [Heng, 2007a] but also to establish new strategies that treat cancer as a genome-based somatic evolutionary disease. At the conceptual level, it is necessary to incorporate many well-established paradigms within the genome theory of somatic cell evolution, including the network theory [Barabási, 2003; Goh et al., 2007], complex adaptive system theory [Holland, 1992; Gell-Mann, 1994; Kauffman, 1995; Coffey, 1998; Vincent and Brown, 2005;

Heng, 2008b; Ao, 2009], ordered heterogeneity [Rubin, 2006], and various evolutionary theories that focus on the patterns and dynamics of micro-evolution [Beerenwinkel et al., 2007; Gerhart and Kirschner, 2007]. Equally important, we need to establish new experimental systems to monitor the overall stress in a genome system, to measure the level of genomic instability, to quantify the degree of heterogeneity in cell populations, as well as to monitor the interactions among subsystems under normal conditions and conditions of stress. Many previously ignored genome-level alterations need to be re-examined including defective mitotic figures (DMF), free chromatin, and chromosome fragmentation [Heng et al., 1988, 1992; Stevens et al., 2007; Ye et al., 2007], and additional genomic methods to analyze both individual cells and cell populations are urgently needed. Effort is also needed (including mathematical modeling and computer simulation) to study the status of genome system dynamics, and to monitor the transition of the different phases or phenotypic hallmarks essential for cancer progression and medical interventions [Quaranta et al., 2008]. A special emphasis needs to be placed on determining which level of genetic or epigenetic heterogeneity should be a priority. There is now an urgent need to test the hypothesis that genome-level alterations rather than variations at lower genetic and epigenetic levels are the most important in cancer evolution as the genome-level changes are the basis of macro-evolution [Heng, 2009]. One of the immediate implications of the genome theory of cancer evolution is the need to re-examine the strategies of sequencing large numbers of ''cancer genomes.'' The key feature of cancer evolution is macro-evolution-mediated genome replacement, and different cancer cases represent different genome systems which display different karyotypes, there is in fact no so-called cancer genome. The cancer genome cannot be defined when cancer karyotypes are drastically different within tumors and among individual patients. The sequencing project will generate huge amounts of data, which will powerfully demonstrate the evolutionary mechanism of cancer but will offer very limited useful information for cancer treatment and diagnosis. Studying collections of multiple cases of the same cancer in order to search for common mutations is similar to the strategy of treating apples and oranges the same and is seriously flawed [Heng, 2007a]. Some other practical issues are determining how to slow down the evolutionary process of cancer, and how not to unwisely accelerate evolution when applying drug therapy (according to the evolutionary view of cancer, overtreatment likely will have an overall negative effect by accelerating cancer evolution), also how can we use system homeostasis to constrain cancer by working on multiple homeostasis systems in individuals, and is it possible to apply the evolutionary cooperation principle to slow down cancer evolution yet not drastically change the cooperative and competitive relationship between cancer and host (e.g., could cancer be directed to enter into a highly homogeneous phase then constrained by the system homeostasis mechanisms of the patient, or can cancer be directed into a slow growth phase that will not trigger much heterogeneity). Clearly, there are many more questions that need to be addressed and many more avenues to be explored, but first of all, it is essential that we appreciate the evolutionary mechanism of cancer and change our way of thinking.

ACKNOWLEDGMENTS

This manuscript is part of our series of publications on the subject of ''the mechanisms of cancer and organismal evolution.'' We would like to thank Dr. Gary Stein, Dr. Gloria Heppner, and Dr. Donald Coffey for their continuous support and interest in this project. Thanks also goes to Rafe Furst, Yong-Bi Fu, and Adam Wilkins for discussions. This work was partially supported by a grant from the Susan G. Komen Breast Cancer Foundation and SeeDNA Biotech, Inc.

REFERENCES

Ao P. 2009. Global view of bionetwork dynamics: Adaptive landscape. J Genet Genomics 36:63–73.

Armtage P, Doll R. 1954. The age distribution of cancer and a multi-stage theory of carcinogenesis. Br J Cancer 8:1–12.

Aubele M, Mattis A, Zitzelsberger H, Walch A, Kremer M, Hutzler P, Höfler H, Werner M. 1999. Intratumoral heterogeneity in breast carcinoma revealed by laser-microdissection and comparative genomic hybridization. Cancer Genet Cytogenet 110:94–102.

Barabási AL. 2003. Linked: How everything is connected to everything else and what it mean for business, science and everyday life. Plume. Cambridge, Massachusetts.

Bartos JD, Gaile DP, McQuaid DE, Conroy JM, Darbary H, Nowak NJ, Block A, Petrelli NJ, Mittelman A, Stoler DL, Anderson GR. 2007. aCGH local copy number aberrations associated with overall copy number genomic instability in colorectal cancer: Coordinate involvement of the regions including BCR and ABL. Mutat Res 615:1–11.

Bayani J, Selvarajah S, Maire G, Vukovic B, Al-Romaih K, Zielenska M, Squire JA. 2007. Genomic mechanisms and measurement of structural and numerical instability in cancer cells. Semin Cancer Biol 17:5–18.

Baylin SB. 2005. DNA methylation and gene silencing in cancer. Nat Clin Pract Oncol 1:S4–S11.

Beerenwinkel N, Antal T, Dingli D, Traulsen A, Kinzler KW, Velculescu VE, Vogelstein B, Nowak MA. 2007. Genetic progression and the waiting time to cancer. PLoS Comput Biol 3:e225.

Bielas JH, Loeb KR, Rubin BP, True LD, Loeb LA. 2006. Human cancers express a mutator phenotype. Proc Natl Acad Sci USA 103:18238–18242.

Bissell MJ, Kenny PA, Radisky DC. 2005. Microenvironmental regulators of tissue structure and function also regulate tumor induction and progression: The role of extracellular matrix and its degrading enzymes. Cold Spring Harb Symp Quant Biol 70:343–356.

Brentnall TA, Brentnall TA, Crispin DA, Rabinovitch PS, Haggitt RC, Rubin CE, Stevens AC, Burmer GC. 1994. Mutations in the p53 gene: An early marker of neoplastic progression in ulcerative colitis. Gastroenterology 107:369–378.

Brock A, Chang H, Huang S. 2009. Non-genetic heterogeneity—A mutation-independent driving force for the somatic evolution of tumours. Nat Rev Genet 10:336–342.

Cahill DP, Kinzler KW, Vogelstein B, Lengauer C. 1999. Genetic instability and Darwinian selection in tumours. Trends Cell Biol 9:M57–M60.

Cairns J. 1975. Mutation selection and the natural history of cancer. Nature 255:197–200.

Coffey DS. 1998. Self-organization, complexity and chaos: The new biology for medicine. Nat Med 4:882–885.

Collins FS, Barker AD. 2007. Mapping the cancer genome. Pinpointing the genes involved in cancer will help chart a new course across the complex landscape of human malignancies. Sci Am 296:50–57.

Crespi B, Summers K. 2005. Evolutionary biology of cancer. Trends Ecol Evol 20:545–552.

de Grouchy J, de Nava C, Cantu JM, Bilski-Pasquier G, Bousser J. 1966. Models for clonal evolutions: A study of chronic myelogenous leukemia. Am J Hum Genet 18:485–503.

Delcuve GP, Rastegar M, Davie JR. 2009. Epigenetic control. J Cell Physiol 219:243–250.

Dexter DL, Kowalski HM, Blazar BA, Fligiel Z, Vogel R, Heppner GH. 1978. Heterogeneity of tumor cells from a single mouse mammary tumor. Cancer Res 38:3174–3181.

Dolinoy DC, Jirtle RL. 2008. Environmental epigenomics in human health and disease. Environ Mol Mutagen 49:4–8.

Duesberg P, Stindl R, Hehlmann R. 2000. Explaining the high mutation rates of cancer cells to drug and multidrug resistance by chromosome reassortments that are catalyzed by aneuploidy. Proc Natl Acad Sci USA 97:14295–14300.

Duesberg P, Li R, Fabarius A, Hehlmann R. 2006. Aneuploidy and cancer: From correlation to causation. Contrib Microbiol 13:16–44.

Esteller M. 2008. Epigenetics in cancer. N Engl J Med 358:1148–1159.

Fan JB, Chee MS, Gunderson KL. 2006. Highly parallel genomic assays. Nat Rev Genet 7:632–644.

Feinberg AP, Ohlsson R, Henikoff S. 2006. The epigenetic progenitor origin of human cancer. Nat Rev Genet 7:21–32.

Feuk L, Carson AR, Scherer SW. 2006. Structural variation in the human genome. Nat Rev Genet 7:85–97.

Foulds L. 1954. The experimental study of tumor progression: A review. Can Res 14:327–399.

Gatenby RA. 2009. A change of strategy in the war on cancer. Nature 459:508–509.

Gell-Mann M. 1994. The quark and the jaguar: Adventures in the simple and the complex. San Francisco: W.H. Freeman.

Gerhart J, Kirschner M. 2007. The theory of facilitated variation. Proc Natl Acad Sci USA 1:8582–8589.

Glansdorff N, Xu Y, Labedan B. 2009. The conflict between horizontal gene transfer and the safeguard of identity: Origin of meiotic sexuality. J Mol Evol 69:470–480.

Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabási AL. 2007. The human disease network. Proc Natl Acad Sci USA 104:8685–8690.

Gonzalez-Garcia I, Sole RV, Costa J. 2002. Metapopulation dynamics and spatial heterogeneity in cancer. Proc Natl Acad Sci USA 99:13085–13089.

Gorelick R, Carpinone J. 2009. Origin and maintenance of sex: The evolutionary joys of self sex. Biol J Linnaean Soc 98:707–728.

Gould SJ, Eldredge N. 1993. Punctuated equilibrium comes of age. Nature 366:223–227.

Greaves M. 2006. Infection, immune responses and the aetiology of childhood leukaemia. Nat Rev Cancer 6:193–203.

Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, Edkins S, O'Meara S, Vastrik I, Schmidt EE, Avis T, Barthorpe S, Bhamra G, Buck G, Choudhury B, Clements J, Cole J, Dicks E, Forbes S, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jenkinson A, Jones D, Menzies A, Mironenko T, Perry J, Raine K, Richardson D, Shepherd R, Small A, Tofts C, Varian J, Webb T, West S, Widaa S, Yates A, Cahill DP, Louis DN, Goldstraw P, Nicholson AG, Brasseur F, Looijenga L, Weber BL, Chiew YE, DeFazio A, Greaves MF, Green AR, Campbell P, Birney E, Easton DF, Chenevix-Trench G, Tan MH, Khoo SK, Teh BT, Yuen ST, Leung SY, Wooster R, Futreal PA, Stratton MR. 2007. Patterns of somatic mutation in human cancer genome. Nature 446:153–158.

Hahn WC, Weinberg RA. 2002. Modeling the molecular circuitry of cancer. Nat Rev Cancer 2:331–341.

Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. Cell 100:57–70.

Harris H. 2005. A long view of fashions in cancer research. BioEssays 27:833–838.

Heng HH. 2007a. Cancer genome sequencing: The challenges ahead. BioEssays 29:783–794.

Heng HH. 2007b. Elimination of altered karyotypes by sexual reproduction preserves species identity. Genome 50:517–524.

Heng HH. 2008a. The gene-centric concept: A new liability? BioEssays 30: 196–197.

Heng HH. 2008b. The conflict between complex system and reductionism. J Am Med Assoc 300:1580–1581.

Heng HH. 2009. The genome-centric concept: Re-synthesis of evolutionary theory. BioEssays 31:512–525.

Heng H, Chen WY, Wang YC. 1988. Effects of pingyanymycin on chromosomes: A possible structural basis for chromosome aberration. Mutat Res 199:199–205.

Heng HH, Squire J, Tsui LC. 1992. High-resolution mapping of mammalian genes by in situ hybridization to free chromatin. Proc Natl Acad Sci USA 89:9509–9513.

Heng HH, Chamberlain JW, Shi XM, Spyropoulos B, Tsui LC, Moens PB. 1996. Regulation of meiotic chromatin loop size by chromosomal position. Proc Natl Acad Sci USA 93(7):2795–2800.

Heng HH, Liu G, Lu W, Bremer S, Ye CJ, Hughes M, Moens P. 2001. Spectral karyotyping (SKY) of mouse meiotic chromosomes. Genome 44:293–298.

Heng HH, Ye CJ, Yang F, Ebrahim S, Liu G, Bremer SW, Thomas CM, Ye J, Chen TJ, Tuck-Muller C, Yu JW, Krawetz SA, Johnson A. 2003. Analysis of marker or complex chromosomal rearrangements present in pre- and post-natal karyotypes utilizing a combination of G-banding, spectral karyotyping and fluorescence in situ hybridization. Clin Genet 63:358–367.

Heng HH, Stevens JB, Liu G, Bremer SW, Ye CJ. 2004a. Imaging genome abnormalities in cancer research. Cell Chromosome 3:1.

Heng HH, Goetze S, Ye CJ, Liu G, Stevens JB, Bremer SW, Wykes SM, Bode J, Krawetz SA. 2004b. Chromatin loops are selectively anchored using scaffold/matrix-attachment regions. J Cell Sci 117:999–1008.

Heng HH, Liu G, Bremer S, Ye KJ, Stevens J, Ye CJ. 2006a. Clonal and non-clonal chromosome aberrations and genome variation and aberration. Genome 49:195–204.

Heng HH, Stevens JB, Liu G, Bremer SW, Ye KJ, Reddy PV, Wu GS, Wang YA, Tainsky MA, Ye CJ. 2006b. Stochastic cancer progression driven by non-clonal chromosome aberrations. J Cell Physiol 208:461–472.

Heng HH, Bremer SW, Stevens J, Ye KJ, Miller F, Liu G, Ye CJ. 2006c. Cancer progression by non-clonal chromosome aberrations. J Cell Biochem 98: 1424–1435.

Heng HH, Stevens JB, Lawrenson L, Liu G, Ye KJ, Bremer SW, Ye CJ. 2008. Patterns of genome dynamics and cancer evolution. Cell Oncol 30:513–514.

Heng HH, Bremer SW, Stevens JB, Ye KJ, Liu G, Ye CJ. 2009. Genetic and epigenetic heterogeneity in cancer: A genome-centric perspective. J Cell Physiol 220:538–547.

Heng HH, Liu G, Stevens J, Bremer S, Ye KJ, Zhang KZ, Wu GS, Shekhar MP, Wei WZ, Ye CJ. 2010. Molecular targeting leads to the macro-evolution of genome systems (Submitted).

Heppner GH. 1984. Tumor heterogeneity. Cancer Res 44:2259–2265.

Heppner GH, Miller FR. 1998. The cellular basis of tumor progression. Int Rev Cytol 177:1–56.

Holland JH. 1992. Adaptation in natural and artificial systems: An introductory analysis with applications to biology, control, and artificial intelligence. Cambridge, MA: MIT Press.

Huang S. 2002. Histone methyltransferases, diet nutrients and tumor suppressors. Nat Rev Cancer 2:469–476.

Jaffe LF. 2005. A calcium-based theory of carcinogenesis. Adv Cancer Res 94:231–263.

Jones RG, Thompson CB. 2009. Tumor suppressors and cell metabolism: A recipe for cancer growth. Genes Dev 23:537–548.

Jones PA, Archer TK, Baylin SB, Beck S, Berger S, Bernstein BE, Carpten JD, Clark SJ, Costello JF, Doerge RW, Esteller M, Feinberg AP, Gingeras TR, Greally JM, Henikoff S, Herman JG, Jackson-Grusby L, Jenuwein T, Jirtle RL, Kim YJ, Laird PW, Lim B, Martienssen R, Polyak K, Stunnenberg H, Tlsty TD, Tycko B, Ushijima T, Zhu J, Pirrotta V, Allis CD, Elgin SC, Jones PA, Martienssen R, Rine J, Wu C (American Association for Cancer Research Human Epigenome Task Force; European Union, Network of Excellence, Scientific Advisory Board). 2008a. Moving AHEAD with an international human epigenome project. Nature 454:711–715.

Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. 2008b. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321:1801–1806.

Kauffman S. 1995. At home in the universe. The search for the laws of self-organization and complexity. New York, NY: Oxford University Press.

Ke XS, Qu Y, Rostad K, Li WC, Lin B, Halvorsen OJ, Haukaas SA, Jonassen I, Petersen K, Goldfinger N, Rotter V, Akslen LA, Oyan AM, Kalland KH. 2009. Genome-wide profiling of histone h3 lysine 4 and lysine 27 trimethylation reveals an epigenetic signature in prostate carcinogenesis. PLoS One 4(3): e4687.

Klein G, Klein E. 2005. Surveillance against tumors—Is it mainly immunological? Immunology 100:29–33.

Knudson AG, Jr. 1971. Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci USA 68:820–823.

Krizhanovsky V, Xue W, Zender L, Yon M, Hernando E, Lowe SW. 2008. Implications of cellular senescence in tissue damage response, tumor suppression, and stem cell biology. Cold Spring Harb Symp Quant Biol 73:513–522.

Lacoste S, Wiechec E, Dos Santos Silva AG, Guffei A, Williams G, Lowbeer M, Benedek K, Henriksson M, Klein G, Mai S. 2009. Chromosomal rearrangements after ex vivo Epstein-Barr virus (EBV) infection of human B cells. Oncogene 29:503–515.

Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K, Dooling D, Dunford-Shore BH, McGrath S, Hickenbotham M, Cook L, Abbott R, Larson DE, Koboldt DC, Pohl C, Smith S, Hawkins A, Abbott S, Locke D, Hillier LW, Miner T, Fulton L, Magrini V, Wylie T, Glasscock J, Conyers J, Sander N, Shi X, Osborne JR, Minx P, Gordon D, Chinwalla A, Zhao Y, Ries RE, Payton JE, Westervelt P, Tomasson MH, Watson M, Baty J, Ivanovich J, Heath S, Shannon WD, Nagarajan R, Walter MJ, Link DC, Graubert TA, DiPersio JF, Wilson RK. 2008. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. Nature 456:66–72.

Liu ET. 2004. Expression genomics and cancer biology. Pharmacogenomics 5:1117–1128.

Maley CC, Galipeau PC, Finley JC, Wongsurawat VJ, Li X, Sanchez CA, Paulson TG, Blount PL, Risques RA, Rabinovitch PS, Reid BJ. 2006. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. Nat Genet 38:468–473.

Manchester KL. 1995. Theodor Boveri and the origin of malignant tumours. Trends Cell Biol 5:384–387.

Mantovani A, Allavena P, Sica A, Balkwill F. 2008. Cancer-related inflammation. Nature 454:436–444.

Martien S, Abbadie C. 2007. Acquisition of oxidative DNA damage during senescence: The first step toward carcinogenesis? Ann N Y Acad Sci 1119:51–63.

Merlo LM, Pepper JW, Reid BJ, Maley CC. 2006. Cancer as an evolutionary and ecological process. Nat Rev Cancer 6:924–935.

Miasari M, Puthalakath H, Silke J. 2008. Ubiquitylation and cancer development. Curr Cancer Drug Targets 8:118–123.

Moenner M, Pluquet O, Bouchecareilh M, Chevet E. 2007. Integrated endoplasmic reticulum stress responses in cancer. Cancer Res 67:10631–10634.

Nelson CM, Bissell MJ. 2006. Of extracellular matrix, scaffolds, and signaling: Tissue architecture regulates development, homeostasis, and cancer. Annu Rev Cell Dev Biol 22:287–309.

Nowak MA, Michor F, Iwasa Y. 2006. Genetic instability and clonal expansion. J Theor Biol 241:26–32.

Nowell PC. 1976. The clonal evolution of tumor cell populations. Science 194:23–28.

Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA, Jr., Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. 2008. An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807–1812.

Quaranta V, Rejniak KA, Gerlee P, Anderson AR. 2008. Invasion emerges from cancer cell adaptation to competitive microenvironments: Quantitative predictions from multiscale mathematical models. Semin Cancer Biol 18:338–348.

Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, Werb Z, Bissell MJ. 2005. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 436:123–127.

Rubin H. 2006. What keeps cells in tissues behaving normally in the face of myriad mutations? BioEssays 28:515–524.

Schatton T, Frank NY, Frank MH. 2009. Identification and targeting of cancer stem cells. BioEssays 31:1038–1049.

Sharp PA. 2009. The centrality of RNA. Cell 136:577–580.

Shen KC, Heng H, Wang Y, Lu S, Liu G, Deng CX, Brooks SC, Wang YA. 2005. ATM and p21 cooperate to suppress aneuploidy and subsequent tumor development. Cancer Res 65:8747–8753.

Stevens JB, Liu G, Bremer SW, Ye KJ, Xu W, Xu J, Sun Y, Wu GS, Savasan S, Krawetz SA, Ye CJ, Heng HH. 2007. Mitotic cell death by chromosome fragmentation. Cancer Res 67:7686–7694.

Tsao JL, Yatabe Y, Salovaara R, Järvinen HJ, Mecklin JP, Aaltonen LA, Tavaré S, Shibata D. 2000. Genetic reconstruction of individual colorectal tumor histories. Proc Natl Acad Sci USA 97:1236–1241.

Vincent TL, Brown JS. 2005. Evolutionary game theory, natural selection, and Darwinian dynamics. Cambridge, UK: Cambridge University Press.

Vogelstein B, Kinzler KW. 2004. Cancer genes and the pathways they control. Nat Med 10:789–799.

Wilkins AS, Holliday R. 2009. The evolution of meiosis from mitosis. Genetics 181:3–12.

Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA, Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JK, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PV, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B. 2007. The genomic landscapes of human breast and colorectal cancers. Science 318:1108–1113.

Wu Y, Zhou BP. 2009. Inflammation: A driving force speeds cancer metastasis. Cell Cycle 8:3267–3273.

Ye CJ, Lu W, Liu G, Bremer SW, Wang YA, Moens P, Hughes M, Krawetz SA, Heng HH. 2001. The combination of SKY and specific loci detection with FISH or immunostaining. Cytogenet Cell Genet 93:195–202.

Ye CJ, Stevens JB, Liu G, Ye KJ, Yang F, Bremer SW, Heng HH. 2006. Combined multicolor-FISH and immunostaining. Cytogenet Genome Res 114:227–234.

Ye CJ, Liu G, Bremer SW, Heng HH. 2007. The dynamics of cancer chromosomes and genomes. Cytogenet Genome Res 118:237–246.

Ye CJ, Stevens JB, Liu G, Bremer SW, Jaiswal AS, Ye KJ, Lin MF, Lawrenson L, Lancaster WD, Kurkinen M, Liao JD, Gairola CG, Shekhar MP, Narayan S, Miller FR, Heng HH. 2009. Genome based cell population heterogeneity promotes tumorigenicity: The evolutionary mechanism of cancer. J Cell Physiol 219:288–300.

Yosida TH. 1966. Relation between chromosomal alteration and development of tumors. Jpn J Genet 41:439–451.

Zaidi SK, Young DW, Javed A, Pratap J, Montecino M, van Wijnen A, Lian JB, Stein JL, Stein GS. 2007. Nuclear microenvironments in biological control and cancer. Nat Rev Cancer 7:454–463.