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Cancer Progression by Non-Clonal Chromosome Aberrations

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Abstract The establishment of the correct conceptual framework is vital to any scientific discipline including cancer research. Influenced by hematologic cancer studies, the current cancer concept focuses on the stepwise patterns of progression as defined by specific recurrent genetic aberrations. This concept has faced a tough challenge as the majority of cancer cases follow non-linear patterns and display stochastic progression. In light of the recent discovery that genomic instability is directly linked to stochastic non-clonal chromosome aberrations (NCCAs), and that cancer progression can be characterized as a dynamic relationship between NCCAs and recurrent clonal chromosome aberrations (CCAs), we propose that the dynamics of NCCAs is a key element for karyotypic evolution in solid tumors. To support this viewpoint, we briefly discuss various basic elements responsible for cancer initiation and progression within an evolutionary context. We argue that even though stochastic changes can be detected at various levels of genetic organization, such as at the gene level and epigenetic level, it is primarily detected at the chromosomal or genome level. Thus, NCCA-mediated genomic variation plays a dominant role in cancer progression. To further illustrate the involvement of NCCA/CCA cycles in the pattern of cancer evolution, four cancer evolutionary models have been proposed based on the comparative analysis of karyotype patterns of various types of cancer. J. Cell. Biochem. 98: 1424–1435, 2006.

Key words: clonal chromosome aberration (CCA); non-clonal chromosome aberration (NCCA); karyotype; cancer progression and evolution; cancer model

Cancer progression has been generally described as a stepwise evolution driven by a series of gene mutations [Nowell, 1976; Fearon and Vogelstein, 1990; Jackson and Loeb, 1998]. This prevailing assumption has produced a major effort to identify mutated genes and their defined molecular pathways, as well as attempt to establish recurrent genetic patterns of cancer progression. It has been widely claimed that only a few gene mutations are needed to turn a normal cell into cancer [Vogelstein and Kinzler, 1993; Hahn et al., 1999] and it is believed that the genetic patterns of this transition are commonly shared by the same types of cancer and thus can be chronologically mapped out by analyzing large numbers of clinical samples representing various stages of cancer progression. Despite over a decades' long effort resulting in the identification of over 100 oncogenes and 30 tumor suppressor genes, the molecular mechanisms of the majority of cancer types, particularly solid tumors, remains more complex than ever as the causative correlation between specific gene mutations and particular types of cancer is now more uncertain and ambiguous. It is also clear that the key mutations responsible for most cancers are hard to

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find and gene mutations may actually represent only a small part of the complete story, as increasing evidence supports a major contribution by both epigenetic and chromosomal aberrations [Duesberg and Li, 2003; Capp, 2005; Cimini and Degrassi, 2005; Duesberg, 2005; Duesberg et al., 2005; Miklos, 2005; Heng et al., 2006a].

The epigenetic cancer theory has support from the fact that the epigenetic influence is linked to altered gene regulation (both at the global level and at the level of specific oncogenes and tumor suppressor genes) and is particularly linked to genomic instability [El-Osta, 2004; Tlsty et al., 2004; Ferres-Marco et al., 2006]. Strong epigenetic effects can be found at various stages of cancer progression, most notably during the early stages [Jaffe, 2003; Capp, 2005]. Therefore, epigenetic plasticity along with genetic lesions provides a major contribution to tumor progression [Feinberg, 2004; Feinberg et al., 2006].

The chromosomal cancer theory has strong support from the fact that for a majority of cancers, chromosomal aberrations are a core characteristic [Atkin and Baker, 1990; Heng et al., 1997, 2004; Lengauer et al., 1998; Albertson et al., 2003]. Increasing evidence demonstrates that chromosomal aberrations represent early events in cancer progression and are not just the result of late stage alterations [Hanks et al., 2004; Rajagopalan and Lengauer, 2004; Duesberg, 2005; Michor et al., 2005; Shen et al., 2005; Heng et al., 2006a]. A recent surprising finding came from a large scale sequencing project. Using clinical samples as well as cancer cell lines, the entire members of the human protein kinase family were sequenced. For most of the 518 genes that were examined, only a few mutations were detected in the coding regions of the patients' samples [Davies et al., 2005; Stephens et al., 2005]. This is extremely unexpected, given the prevailing view that protein kinases are frequently mutated in human cancer. Most interestingly, in the patient samples that lacked gene mutations, chromosomal aberrations were easily detected [Bignell et al., 2006], suggesting that it is the chromosomal aberrations that are linked to cancer and not the gene mutations. A similar view has gained strong support from various genome sequencing projects demonstrating that it is genome variation and not gene variations that distinguish between different species or individuals and that chromosomal aberrations and not gene mutations that are the major form of genome aberration and variation [Venter et al., 2001; Feuk et al., 2006; Heng et al., 2006a]. It is therefore time for cancer researchers to critically evaluate the conceptual framework of cancer research to answer the most basic questions, such as, is the gene mutation theory, the epigenetic theory, or the chromosomal-based theory, the correct cancer theory? Is the pattern of cancer evolution a chronological stepwise process that can be traced by molecular or karyotypic comparisons or is it something different, such as a non-linear stochastic process that is difficult to trace [Schwab and Pienta, 1996].

To address these questions, one needs to illustrate various molecular mechanisms of how gene mutations, epigenetic effects, and chromosomal changes occur and then determine how they drive cancer progression. In particular, the relationship between gene mutations, epigenetic effects, and chromosomal aberrations and the pattern of progression within the context of cancer evolution needs to be addressed. Opposing views both supporting and challenging the mutation-based theory and chromosomal-based theory have been previously reviewed [Jackson and Loeb, 1998; Hahn and Weinberg, 2002; Gibbs, 2003; Jaffe, 2003; Soto and Sonnenschein, 2004; Capp, 2005; Duesberg et al., 2005; Miklos, 2005; Heng, 2006]. In this perspective, we have focused on some of the recent exciting discoveries regarding the pattern of karyotypic changes during cancer progression that support the view of chromosomal/genome aberrations as a prime underlying cause of cancer [Heng et al., 2006b]. Specifically, we will discuss the importance of non-clonal chromosome aberrations (NCCAs) as well as the interplay between clonal chromosome aberrations (CCAs) and NCCAs that defines karyotype dynamics and cancer progression. These new insights connect the elements of chromosomal/genome variation/aberrations with genomic instability and cell population diversity that provide new explanations for the differential patterns of cancer evolution.

PATTERNS OF CANCER PROGRESSION ARE DRASTICALLY DIFFERENT AMONG VARIOUS CANCER TYPES

Cancer is a disorder that displays "out of control growth" phenotypes. The understanding

that various types of cancers display different genetic and phenotypic patterns of disease progression is of ultimate importance when attempting to define parameters for diagnosis and treatment of a specific type of cancer. However, despite the well known fact that hematologic cancers and solid tumors are significantly different and that the former only accounts for a small portion of the total cancer cases, the conceptual framework for cancer is primarily based on models of hematologic cancers.

According to the genetic profile for hematologic cancers, there are usually karyotypic signatures that display recurrent genetic aberrations and subtypes of cancer [Rowley, 1998; Johansson et al., 2002; Bullinger and Valk, 2005]. As a result, the progression of cancer can be staged using recurrent karyotypes or even by using gene expression profiles. Chronic myeloid leukemia or CML, for example, can be characterized as a t(9; 22), which is the common initiating step detected among CML patients. This particular fusion gene will then trigger a stepwise progression evidenced by a sequential karyotypic change. Based on this clear-cut model of cancer progression, it has been hypothesized that cancer progression is driven by accumulations of temporal mutations occurring in a continuous linear pattern of cancer evolution [Nowell, 1976; Fearon and Vogelstein, 1990].

This type of stepwise progression has not been detected in most solid tumors. Initiating factors for solid tumors are varied and the overwhelming genetic aberrations that are detected are not the recurrent types giving the impression that cancer initiation and progression in solid tumors is more or less random [Heppner and Miller, 1998; Miller and Therman, 2003]. Clearly, heterogeneous pathways are involved at a much higher level in solid tumors than in blood cancers.

There appears to be many factors contributing to the distinction between blood cancers and solid tumors. By further comparing hematologic cancers and solid tumors from an evolutionary context, the key difference between continuous and discontinuous patterns of karyotypic evolution seems to be the differential mechanisms of evolutionary changes including mutation types and the impact of genetic drift on both cancer systems. We have discussed a few factors that have significant influence over the mechanisms of evolution, such as the size of the tissue system (the blood system represents a large cell population vs. small population of particular regions of the solid tissue system), the special features of a cell population (altered blood cells that are moveable within the entire system vs. altered cells that are fixed and isolated in solid tissue), the timeline of specific events (recurrent genetic alterations in a blood system frequently occurs early in a cell lineage vs. sporadic recurrent changes that often occur late due to the accumulation of genomic changes caused by instability in solid tissue), and the gene types involved (gatekeeper or caretaker genes) [Heng et al., 2006c]. This analysis clearly groups hematologic cancer and solid tumors into different categories that explain the drastically different patterns of cancer evolution. In addition, the different types of solid tumors can be further divided into different subtypes according to the gene types involved and the penetration of mutated genes. All of these analyses suggest that there should be different evolutionary models for different types of cancers, as the basic elements required for the cancer evolutionary process to commence and proceed are different.

PATTERNS OF SOLID TUMOR PROGRESSION ARE DRIVEN BY NON-CLONAL CHROMOSOME ABERRATIONS

As predicted in the previous section, the patterns of solid tumor evolution may be significantly different as many parameters are clearly different between blood cancers and solid tumors. The blood cancers can be more or less described as a linear progression with gradual change in genetic patterns, which can be traced by karyotypic analysis or molecular profiling [Rowley, 1998; Johansson et al., 2002; Bullinger and Valk, 2005]. Solid tumors display high levels of karyotypic heterogeneity and the progression patterns are difficult to trace. We hypothesize that the non-linear pattern of progression is likely the dominant pattern in solid tumor evolution. To prove this viewpoint, various factors have been introduced to destabilize the genome including the dysfunction of genes that maintain genetic integrity, the introduction of onco-proteins, and the application of carcinogens. To systematically analyze the patterns of the chromosomal aberrations, multiple color spectral karyotyping (SKY) was applied to score large numbers of individual

mitotic figures [Heng et al., 2001; Ye et al., 2001]. The use of molecular cytogenetic tools like SKY to profile individual cells within a cell population rather than using the average profile generated from cell mixtures are essential to detect the NCCAs [Ye et al., 2006]. As shown in Figure 1, the SKY assigns a unique single color to each individual normal chromosome and mixed colors detected along a chromosomal arm indicates translocation events. Both the numerical and structural aberrations are scored and the karyotypic patterns are then compared. Interestingly, the common initial responses to all these internal or external challenges are the increased frequency of NCCAs, and not the increase of recurrent types of CCAs; Furthermore, when the CCAs were detected during the late stages, the unrelated types of CCAs are often generated in a seemly random fashion, suggesting that the stochastic nature of chromosomal changes are the direct result of these challenges and could serve as the basis for non-linear progression. In all the systems that we examined, it was observed that the higher the degree of either internal or induced genome instabilities, the higher the frequency of NCCAs and the more diverse CCAs if CCAs can be formed later on. Thus, the degree of NCCAs directly indicates the overall genomic instability and the degree of variation in the CCAs or karyotypic heterogeneity that causes cell population diversity [Heng et al., 2004]. In other words, when high levels of NCCAs are detected, it means that the particular cell population is unstable. When specific CCAs are established and are coupled with low frequencies of NCCAs, it means that the particular cell population is stable. Judged by the fact that all cells in Figure 1 share the same set of several altered chromosomes, this cell population is very stable. According to our data, for most of the established immortal cell lines, the CCAs are relatively stable with low frequencies of NCCAs. However, during the crisis

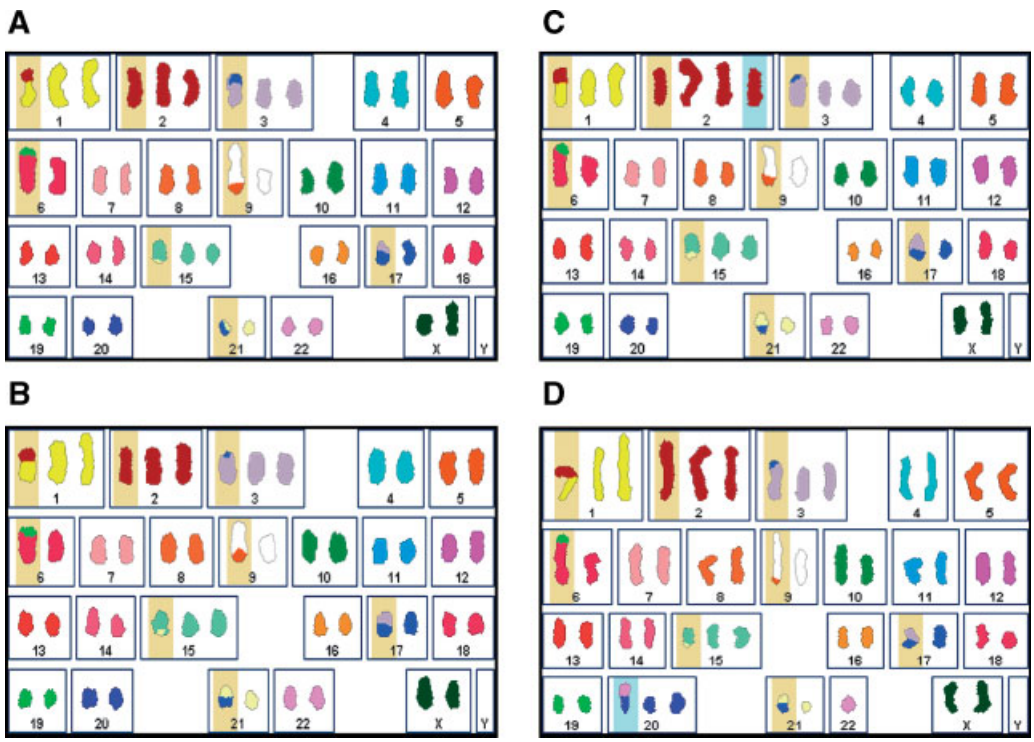

Fig. 1. SKY karyotypes of four tumor cells isolated from a xenograft mouse model of human breast disease illustrating CCAs and NCCAs. Images (**A** and **B**) represent the clonal chromosome aberrations or CCAs as the two cells share identical sets of altered chromosomes (both structural and numerical alterations) highlighted by light yellow coloration. These altered chromosomes clearly belong to the recurrent aberration category. Images (**C** and **D**) represent both CCAs and simple types of non-clonal chromosome aberrations (NCCAs). In addition to the CCAs identical to (A) and (B), there are additional chromosomal changes highlighted by light blue coloration that are not shared by other cells. These non-clonal alterations belong to the non-recurrent aberration category. Image (C) shows one structural NCCA (this cell gained an additional translocation involving chromosomes 20 and 22) and (D) shows one numerical NCCA (this cell gained an additional copy of chromosome 2).

stage prior to the establishment of immortalization, the NCCAs reach the highest level where only a few or even no CCAs could be detected (data not shown). Clearly, the later selected dominant CCAs that follow immortalization are established and formed at random and originate from one selected NCCA among many possible combinations of aberrations [Heng et al., 2006b].

According to the standard practice, NCCAs, particularly the simple types, have been considered to be non-significant genetic background [Mitelman, 2000; Albertson et al., 2003] and have been largely ignored [Heng et al., 2004, Heng et al., 2006a]. Following the establishment of the importance of NCCAs, we studied the pattern of NCCAs during cancer progression using an in vitro model of immortalization. Using SKY to trace individual cells within representative populations that are stage specific during the immortalization process, we have shown that the key feature of karyotypic evolution during immortalization are the dynamic stochastic NCCAs and their interplay with different CCAs, coupled with various degrees of genomic instability. Our data can be summarized by the following points:

1. The initial genomic changes (represented by NCCAs) occur at random when the genome is unstable. The degree of stochastic changes can reach extremely high levels right before the crisis stage (karyotypic chaos) where none of the cells are the same in a given population. This surprising observation challenges current methodologies of studying cancer cells that have assumed that the majority of the cells are the same and that heterogeneity represents a minority of the cell population.
2. The evolutionary process of cancer can clearly be divided into two phases as judged by the karyotypic patterns: the discontinuous phase (marked by elevated non-clonal events, NCCAs and transitional clonal events, transitional CCAs) and the stepwise continuous phase (marked by stepwise clonal evolution, stable CCAs). The key event that separates these two phases is the cell crisis stage. Different from previous models of cancer evolution, our data demonstrate that stochastic karyotypic aberrations rather than sequential recurrent aberrations are the basis for early evolution. The unpredictable genotypes of cancer are caused by the stochastic nature of the initial phase of cancer progression.
3. The degree of genomic instability can be monitored by the degree of stochastic chromosomal changes. In particular, we have found that NCCAs are important indicators of chromosomal instability. Conversely, we have found that clonal aberrations do not correlate with genomic instability.
4. Karyotypic heterogeneity is caused by stochastic NCCAs and their interplay with CCAs. By comparing the frequency and types of NCCAs and CCAs during the immortalization process, we have noticed that the NCCA/CCA cycle corresponds well with cancer progression. When NCCAs dominate, a cell population is within an unstable "struggling to survive" phase coupled with high levels of genomic instability and increased genomic heterogeneity. When CCAs dominate, a cell population is within a relatively stable "growth" phase displaying greater stability and dominant pathways. Interestingly, cancer progression occurs through multiple cycles of NCCAs/CCAs, with the cycle also recurring in response to drug treatments [Heng et al., 2006b; Heng unpublished observations]. Based on our analysis, we predict that multiple cycles of NCCAs/CCAs are needed for a normal cell to first turn cancerous and then to further progress into advanced cancer cells. One such cycle was illustrated in Figure 2. A particular CCA such as CCAx can be formed stochastically from NCCAs during the cancer evolutionary process. After a certain time period of growth, the CCAx population will then be replaced by the NCCA population, until the next stage where new CCA populations, such as CCAy form and became dominant. When both the genome and environment are stable, the CCAx population and CCAy population often share some karyotypic signatures and the transition takes a much longer period of time. When the genome is unstable regardless of whether it is due to internal factors or induced, the CCAx and CCAy populations are usually drastically different demonstrating the stochastic nature of karyotypic evolution. NCCAs provide the material and the opportunity

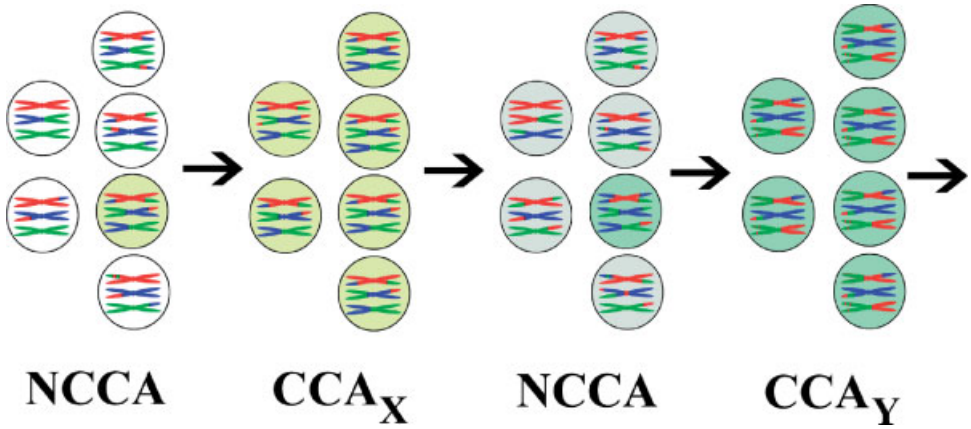

Fig. 2. A diagram illustrating the model of stochastic interplay of NCCAs and CCAs that drives cancer progression: One single cycle of the NCCA/CCA interplay is illustrated and this dynamic interaction forms the centerpiece of tumor cellular progression occurring many times throughout the entire evolution process. The timeline of cancer progression is represented by four arrows. First, there are cells with NCCAs that are highlighted by white coloration and various combinations of altered chromosomes. Second, one of the cells with a particular NCCA forms a cell population with a defined CCAx (light yellow color). Third, this population of cells is replaced by another cell population with NCCAs (light grey) as a result of a changed environment and its associated selection pressures. Fourth, one of the newly formed NCCAs develops into a cell population with a new CCAy (green). The new cell population containing the CCAy can become dominant if the genome and environment is stable, otherwise, another cycle of NCCA/CCA will soon follow. CCAx and CCAy are illustrated by different colors indicating different chromosomal combinations as CCAs often do not share related chromosomal aberrations thus demonstrating that karyotypic progression is not continuous for solid tumors. The discontinuous patterns of CCAs seen in solid tumors are caused by the intermediary role played by NCCAs. The formation of CCAx and CCAy are the result of apparent random selection indicated by the use of X and Y.

for cancer evolution to occur and CCAs are the end products of a given stage of evolution as defined by a specific selected NCCA and its environment. For a majority of solid tumors the importance of a given CCA is limited in terms of tracing a common path of progression, as late stage CCAs are often not shared by different tumors of the same cancer type. The complexity of CCAs, however, is of value as it reflects the clonal diversity and the selective results of NCCAs. NCAAs and their dynamic interplay among various combinations of NCCAs/CCAs is what drives and shapes cancer progression. Our view agrees with the observations generated from a systematic analysis of the literature that the cancer karyotypes in late phases display more heterogeneity, as karyotypic evolution is a highly disorganized process during the late stages resulting in the disintegration of pathways [Hoglund et al., 2002]. Such increased karyotypic heterogeneity contributes to the loss of long-range correlations that is also reflected by increased NCCAs and newly emerging CCAs.

STOCHASTIC CHANGES ARE UNIVERSALLY DETECTABLE AT DIFFERENT LEVELS OF GENETIC ORGANIZATION

Since altered chromosomes have a direct impact on molecular pathways, stochastic NCCA/CCA combinations result in the often detected heterogeneity of molecular pathways. For many gene knockout mouse models, various tumors display different karyotypes and are often involved with different molecular pathways [Sharpless, 2001]. Using AML as an example, the correlation between specific karyotypic aberrations and defined molecular pathway changes has been successfully demonstrated recently with expression microarrays [Bullinger and Valk, 2005]. For all the major CCAs detected from a patient population, a given gene expression profile was established. Based on this information, it would be logical to suggest that even though it would be difficult to experimentally demonstrate, stochastic NCCAs should generate different pathways in a stochastic manner.

In fact, NCCAs are non-recurrent aberrations and represent only one form of stochastic genetic change. It is known that the gene mutations

and epigenetic effects occur stochastically as well [Jaffe, 2003; Capp, 2005]. Given the fact that many genes and gene combinations are involved in a specific cancer, we anticipate that for solid tumors, it would be a challenge to obtain consistent expression profiles even without the influence of chromosomal level changes, particularly in the early stages of progression.

Universally existing multiple levels of stochastic genetic aberrations create a challenge for cancer researchers, but it is this precise characteristic that allows cancer cells to succeed by escaping surveillance systems and surviving drug treatments. From a cancer evolutionary point of view, the basic requirement for cancer evolution to occur is to introduce genetic variation, regardless of whether these variations come from gene mutations, epigenetic effects, or chromosomal aberrations (we will discuss in the next section that genetic aberrations at the chromosomal level are actually the most influential). In a minority of cancer cases, specific gene mutations can initiate cancer evolution, as exampled by CML. For the majority of cancer types, however, genomic instability is responsible for genetic variations. When genomes are unstable, caused by either internal or external factors, stochastic genomic changes (including genetic and epigenetic changes) will occur. When the instability reaches a certain level, the altered cell population will form cycles of stochastic/specific genomic changes and this reflects on the balance of growth and diversity of a population, illustrated by NCCAs/CCAs cycles. As soon as the evolutionary process has been initiated other genetic or environmental factors will further act as agents to alter the speed of evolution. After all, evolutionary selection is based on selected phenotypes and many different combinations of genome types can achieve the same phenotype, namely, the out of control growth that defines cancer cells.

GENOME LEVEL ABERRATIONS REPRESENT THE MOST EFFECTIVE FORM OF GENETIC VARIATIONS

To acknowledge our view that cancer progression is driven by stochastic NCCAs, one needs to accept the concept that genome level aberrations represented by chromosomal aberrations are more dominant than any other forms of genetic or epigenetic variation for the majority of cancer types. We have recently presented a few viewpoints to support that genetic aberrations at the genome/chromosome level are more significant than at the gene level [Heng et al., 2006a]. Briefly, genome variation usually involves gene and epigenetic variation on a much larger scale and as a result gene combinations generated by genome rearrangement cannot be achieved by changes solely at the gene level within a comparable period of time. In addition, different karyotypes have been used to define different species [King, 1993], and this point has gained a great deal of support from recent genome sequencing projects that it is genome rearrangements and their subsequent evolution, and not just specific genes that are responsible for the formation of different species [Navarro and Barton, 2003].

The degree of genetic variation determines the pattern of evolution and chromosomal aberrations are more significant than gene mutations, not only due to the fact that stochastic chromosomal combinations can "amplify" or "reduce" any impact that might be caused by a given gene mutation, but also due to the unique role of genome organization of genes to coordinate gene function. Most importantly, the infinite karyotypic combinations suggested by our studies is the main driving force for generating genomic heterogeneity in cells providing a substrate for cancer evolution.

Another point that needs to be mentioned is that the epigenetic contribution in cancer arises through gene mutations and particularly through chromosome aberrations. For cancer evolution to proceed, genetic variations must be involved. The tricky part of the epigenetic impact are the stochastic and reversible changes that can initiate various pathways or change the balance of competition among various factors, and finally cause genetic variation to occur. In this sense, epigenetic effects either can be considered as a prototype of genetic change or serve as a "passage" to introduce variation. An example is the linkage between epigenetic change and genomic instability [El-Osta, 2004; Tlsty et al., 2004]. It is known that increased genomic instability can cause genetic variation either by gene mutations or by chromosomal aberrations or both. When the damage is done, we may or may not be able to determine the causative factors, such as the original epigenetic effects. Based on the consideration that epigenetic effects are usually involved with global gene expression, and the degree of

genetic variation is significantly higher at the chromosome level, thus, we conclude that the epigenetic effect will have a greater impact at the chromosome/genome level. In addition, different from gene mutations, the epigenetic effects often can quickly impact many cells providing variations at the cell population level. Of course, an integrated cancer model is needed to link gene mutations, epigenetic effects, and genome aberrations. As we have discussed [Heng, 2006], such integration will not generate a universal model to cover all the various types of cancer and instead, a series of models needs to be generated to cover different cancer types.

A CALL FOR NEW INTEGRATED MODELS OF CANCER EVOLUTION

The patterns of cancer progression are drastically different among various cancer types and the patterns of cancer evolution are defined or influenced by many essential elements, such as the size of a cell population, the architectural constraints of altered cells within a given tissue type, the degree of penetration of mutated genes or chromosomal aberrations, and the timeline of a "hit" of a dominant pathway coupled with either an aggressive phenotype or by significantly increased overall genome instability, or both. Based on the newly discovered relationship between overall genomic instability and karyotypic heterogeneity, we would like to present a model consisting of four types of cancer progression. This model of cancer evolution includes NCCA/CCA interplay and various basic evolutionary elements. It should be pointed out that the classifications described below serves as examples of this integrated model. As defined by the heterogeneity nature of cancer, many more subtypes could be summarized.

Model 1: A Classic Stepwise Evolution Model Suitable for Cancers With Linear Types of Progression

In this cancer type, both cancer initiation and progression is clearly defined by a specific recurrent genetic aberration, such as specific oncogenes/tumor suppressor genes, or a specific chromosomal aberration leading to fusion genes or a particular combination of genes. From an evolutionary viewpoint, the key feature is a continuous stepwise pattern of evolution at the genotype level. The stage or subtypes of disease corresponds to a specific karyotypic pattern or gene expression profile that can be used in clinical applications [Bullinger and Valk, 2005]. The formation of such a pattern requires that both a large cell population and the environment need to be relatively stable, and the specific pathway defined by recurrent genetic changes is dominant with a clear-cut growth advantages. Examples can be found in blood cancers and children's solid tumors. CML represents the best example of this category, with its karyotypic evolution illustrated by a series of predictable CCAs.

Even within this category, there are subtypes likely caused by NCCAs. In the early stages, signature CCAs dominate, and sequential CCAs are often detected in the course of cancer development. During this process, genomes became more unstable coupled with elevated frequencies of NCCAs that introduce heterogeneity. Such heterogeneity then becomes dominant within the cell population and more diverse CCAs are detected during the late stages. This is seen during the blast phase of CML where increasing NCCAs and variable CCAs are often detected. It would be interesting to investigate whether the degree of clonal diversity correlates to the speed of cancer progression among patients and their response to treatment, as population diversity favors both cancer progression and dry resistance.

Model 2: A Mixed Stepwise and Stochastic Evolution Model That Has Partially Traceable Patterns of Progression

For this type of cancer, there is a clear connection with recurrent genetic changes marked by inherited genes that result in some overall trend, but there is no clear-cut pattern of evolution due to the involvement of genomic instability. Initiation can occur by inherent oncogene/tumor suppressor genes, or by specific chromosomal aberrations, or a specific pathway could be activated that could lead to a stepwise progression. However, as these defective genes also involve genomic stability, they cause stochastic changes with a certain degree of non-linear progression. The combinational effects of both stepwise and stochastic changes thus define the pattern for this cancer type as more or less traceable with a certain degree of heterogeneity.

Various subtypes can be found in this category, depending on the event timeline when

genomic instability occurs. When instability occurs early during cancer progression, more heterogeneity will be detected; when instability occurs at a late stage, however, less heterogeneity will be detected. Overall, commonly shared genetic aberrations can be detected from a majority of the patients but the pattern of progression varies.

Model 3: A Stochastic Evolution Model That Is Defined by Genes That Are Defective in Maintaining Genome Integrity

For this type of cancer, cancer progression is initiated by defective genes causing genomic instability. As demonstrated, many defective genes in this category will interfere with the chromosomal structure/behavior/function. Except for some dominant fusion genes, most of the chromosomal aberrations are of the stochastic type and the chromosomal changes display a high degree of heterogeneity among cell populations, which drives the non-linear patterns of karyotypic evolution that are seen in this type of cancer.

Various types of cancer can be grouped under this category. The common requirement of this category is the early involvement of genes that cause genomic instability. These defective genes can affect different tissue types. Different cancers in this category share the same mechanism of instability causing initial karyotypic changes and then the stochastic karyotypic aberrations drive the evolutionary process. Since stochastic NCCAs are the driving force in these types of cancer, a high degree of genotypic and pathway heterogeneity is the signature of this category. Due to the fact that various pathways are randomly hit and selected, it is difficult to detect recurrent genetic changes. Even though the main feature is stochastic karyotypes and molecular pathway changes, there are possibilities of genetic convergence due to tissue-specific environments and the selection of similar pathways defined by oncogenes or tumor suppressor genes occurring during the late stages of progression.

Model 4: The Stochastic Evolution Model Is Initiated by Variable Factors Including Genetic Variation, Epigenetic Effects, and Environmental Influences

The majority of cancers belong to this category and in particular the sporadic cancers. It is known that in sporadic cancers, there seems to be variable causative genetic, epigenetic, and/or environmental factors involved and each factor has a limited effect on contributing to cancer progression within the entire population of cancer patients. This category has a high degree of genetic variation and is different from previous categories where defective genes dominate. This category features genes that are often less dominant and display low penetration within the population [Hunter, 2005]. Some defective genes seem to lack clear linage to specific oncogene/tumor suppressor genes or instability genes, but can introduce variation or disorganization, which is needed for cancer evolution. Epigenetic effects are another major source of variation [Capp, 2005; Esteller, 2006]. Interestingly, environmental effects such as carcinogenic insults challenge many cells rather than cause mutation in a few cells [Jaffe, 2003]. The epigenetic effects seem to represent the first line of reaction towards environmental changes. Regardless of where the variations come from, the different types of variation clearly serve as initiation factors in the cancer evolution of this category. The next step in cancer progression in this category is a stochastic one and will involve a combination of various pathways. It could include dominant pathways from categories 1 to 2, but the majority of them would be expected to be similar to category 3, as genomic instability is a common and key signature element, which is responsible for the heterogenic patterns of cancer evolution.

Most hematologic cancers and children's solid tumors belong to the first two categories and most adult solid tumors belong to second two categories. In general, genes that belong in the first category are typically gatekeeper genes, and the genes that belong to the third category are typically caretaker genes [Levitt and Hickson, 2002]. However, there are some overlapping stages or patterns among different models. Even in blood cancers, certain cases could be classified into other categories as a high degree of instability is clearly involved. On the other hand, some cases from category 4, even though the initiation factors are stochastic epigenetic effects caused by carcinogenic insult, specific known dominant oncogenetic pathways could be actived by shear chance and the following progression patterns can thus be detected, giving the wrong impression that these cases are typical of categories 1 or 2.

When progressing from category 1 to category 4, the genetic or molecular predictability decreases as the degree of stochastic variation increases. For many category 1 cases, the genotype and phenotype correlate providing predictability for diagnosis and treatment. Most cancer cases from categories 3 to 4, particularly when genomic instability pathways are involved during early stages of cancer progression, it is very difficult to trace the initiation factor and it becomes less meaningful to the treatment of cancer, as the pathways detected from late stages are likely completely different from the initial causal factors, and the dynamics of the karyotypic evolution is so diverse resulting in each case being genetically different. Similarly, the genetic pattern is less important when the cancer genome is unstable enough where certain phenotypes will be selected for their "out of control growth."

Since the majority of cancer is initiated by multiple factors displaying non-linear patterns of cancer evolution; and as the genomic instability-defined chromosome or genome aberration plays a central role in cancer evolution; and as NCCAs are generated from stochastic chromosomal or genome aberrations, we conclude that for the majority of cancers, initiation and progression are derived from NCCAs.

CONCLUDING REMARKS

So far we have argued that the stepwise evolutionary pattern of cancer progression can only be detected from certain limited types of cancer, and in the majority of solid tumors, stochastic NCCAs are the driving force. To agree with our viewpoint, one needs to accept the following important facts and assumptions: First, stochastic genomic changes are more frequently involved in cancer progression than recurrent types of changes. Using an in vitro cell culture model, our recent data have provided the evidence that indeed NCCAs (representing stochastic changes) and not CCAs (representing recurrent changes) are linked to solid cancer progression. If we include the collective effects of multiple levels of genetic and epigenetic effects, the degree of stochastic changes will be further increased. Recent examples can be found from the collective effect of aneuploidy and tumor suppressor genes [Shen et al., 2005], as well as epigenetic effects and mutations [Ferres-Marco et al., 2006]. Second, genetic or epigenetic changes at the chromosomal level are much more dominant than at the gene level. It is true that stochastic genetic and epigenetic variations can be detected at various levels including at the gene mutation level, however, genome level aberrations have a much greater impact and involve many more genes [Heng et al., 2006]. Our arguments are additionally supported by the arguments of others [Duesberg, 2005; Miklos, 2005], and by the fact that most cancers involve chromosomal instability [Atkin and Baker, 1990; Lengauer et al., 1998; Albertson et al., 2003] and chromosomal aberrations thus represent universal features of cancer. This is not true for a given gene mutation. Third, the central role of stochastic chromosomal aberrations and not recurrent chromosomal aberrations or gene mutations, is a much more plausible situation and the karyotype patterns are a better fit in accordance with an evolutionary system. It is known that genetic variation occurs randomly during the evolutionary process. As long as the genome is unstable as a result of chromosomal changes, defective genes, epigenetic changes, environmental challenges, or the combination of all of these factors, stochastic genomic changes will be induced and initiate the evolutionary process. Following the initiation period, other factors can serve as promotion factors changing the speed of evolution. When initiating and promoting factors are available, cancer evolution will precede stochastically using different pathways, slower or faster, one way or another.

The above analysis is essential to understand some of the key findings in the field of cancer research and provide a guide for future cancer research as well as clinical applications. For example, when high levels of dominant oncogenes are artificially expressed under experimental conditions (either by cell transfection or transgenic mice approaches), it is relatively easy to activate certain pathways that are defined by these over-expressed oncogenes. This situation may not actually occur at all in the natural state where there is no such strong expression of these manipulated oncogenes and the activation of particular pathways are more dynamic and stochastic. In addition, when commonly used animal models are utilized, the correlation between specific genetic aberration and the phenotype would tend to be more homogenous than in a real patient population, as the genetic background is drastically less

pure in patients than in well controlled animal models. The heterogeneity of the background will certainly involve different combinations of pathways in a stochastic manner.

One key aspect of our analysis is that various types of cancer are distinguished according to their evolutionary patterns. This information is essential for accurate diagnosis as well as treatment. For instance, a category one type cancer, microarray analysis can generate expression profiling useful to classify the stages and subtypes of genome types, as specific translocations are linked to specific signatures [Valk et al., 2004]. The same approach might face a great deal of uncertainty in solid tumor profiling due to the stochastic patterns of cancer evolution. On the other hand, however, the difficulty in predicting NCCAs could also be used as a tool in early diagnosis. As increased frequencies of NCCAs occur far earlier than any detected CCAs, the initial increase in NCCAs could be effectively used to monitor overall genomic instability [Heng et al., 2006a,b]. Similarly, the degree of CCAs rather than specific CCA type is useful for monitoring clonal diversity Our analysis of cancer in the context of cancer evolution also emphases the importance of the prevention of cancer, as it would be a much better strategy to prevent the genome from becoming unstable rather than treat the genome after it has become unstable. As for treatment considerations, the key to blocking specific oncogenic pathways and at the same time stabilize the genome is to reduce further genetic variation that will continue to further push towards cancer evolution. Caution should be taken when considering treatment strategies that will drastically destabilize either a normal or a cancer genome, as the evolutionary consequences could be dire for a patient.

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