The fabric of cancer cell biology—Weaving together the strands

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It is always attractive and seductive to describe any time in research as being one of transition, where the knowledge and insights of past work and the tools of our trade coalesce to fundamentally alter our concepts, our approaches, and our capacity to make progress. In truth, it is often easy to argue the case for such a transition, or even a transformation, but it is exceedingly difficult to prove. Whether we are at a profound transition in cancer research is an interesting question. The answer may be yes, but it may be more of a reflection of a long and ongoing transition rather than a unique inflection point. That said, I believe that we are at a point of emerging synthesis, new directions, and the emerging possibility that the science of cancer research could actually inform and transform the approach to cancer in patients and in populations. I am taking the liberty of a perspective to take a more conceptual overview of the types of questions being asked, to discuss how the enormous amount of knowledge gained over the past decades can be organized, and to point to the challenges (conceptual and technical) that lie ahead. I focus on the process of organization and synthesis because it is our emerging ability to bring a higher level of order to how we think about cancer, pulling together the flood of information garnered by thousands of researchers over decades, that, in part, provides a useful description of the beginning of the twenty-first century as a transition period in cancer research.

There is a bit of a paradox here. On the one hand, our emerging ability to synthesize information and to bring some simplifying order to cancer defines one aspect of this transition time, while on the other hand, as I will discuss, it is the need for and ability to begin to address cancer more directly in terms of its complexity that will characterize cancer research as we move forward. In the first, we are bringing together immense amounts of information gathered through a very successful reductionist approach to the study of cancer cells. In the second, we will need to address the study of cancer as very complex systems whose machinery needs to be understood in multiple specific contexts—of interacting molecular networks, of the cellular lineages involved, and of the individual in whom the cancer has developed. With the first, we are attempting to answer the question, "What is the molecular nature of cancer?" With the second, we are attempting to answer surprisingly subtle and complicated questions, such as "How many different cancers are there?" and "How do the molecular components of cancer play out in causing a specific disease in a particular individual?"

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The nature of cancer—A satisfying synthesis

The long and frustrating history of cancer research over the first seven decades of the twentieth century seemed to provide little in the way of unifying concepts. Attempting to connect models for the nature of cancer with insights gained from the study of the causes of the disease proved confusing. Causes ranged from radiation to chemicals to infectious agents to heredity. It was the growing recognition that DNA was the crucible through which the external and internal environments, experience and heredity, came together that provided our first great unifying view (Bishop and Weinberg, 1996). We now know that cancer is an acquired genetic disease in which a single clone of cells and its progeny accumulate heritable changes that result in the cellular phenotype of cancer. This realization set the stage for the modern era of cancer biology. Our goals have been clear: (1) to define the mechanisms by which cells acquire and fix genetic changes, (2) to identify the specific changes, (3) to understand the functions and malfunctions of the genes involved, and (4) to elucidate the roles of specific changes in either the development of and/or the behavior of cancer cells.

The genetic nature of cancer has now been amply demonstrated—first, by the remarkable and productive application of human genetics to cancer; second, by the successful production of cancer in mice via the specific engineering of alterations in the orthologous genes to those identified in humans; and third, by the validation of the critical role of defined pathogenetic changes in human cancer by the development of drugs that work by targeting such genetically defined cancer-specific altered molecular machines. The best example of this is the recent experience with an inhibitor of the abl kinase in CML (Mauro et al., 2001). This work capped a long (4 decades) story that first identified the Philadelphia chromosome as a characteristic heritable change in the structure of the genome in CML cells. The nature and function of the product of this genomic change was identified as the bcr-abl fusion, which constitutively activates the abl tyrosine kinase, and the activity of this kinase was shown to be essential for the proliferation and survival of the leukemic cells. Next, the introduction of the fusion gene into

the mouse produced a CML-like leukemia. Finally, the use of an active inhibitor of the abl kinase in patients induced remission in the chronic phase of the disease. The failure to sustain remissions in the accelerated or blast phase is associated with specific molecular mechanisms of resistance to the ability of the drug to inhibit the kinase (Hochhaus et al., 2001; also see Druker, 2002 [this issue]).

A further typology of the nature of cancer

I think it unlikely that we will replace our current view of the nature of cancer as a disease of heritable changes in a single clone of cells. However, we can refine the question of the nature of cancer into a more structured typology, through which we can answer the question of the nature of cancer along three lines. For each of these, I will present a description of a simplifying commonality across cancers, but also address the great challenges that these common themes face in light of both the complexity of cancer and of the recognition that the manifestation of cancer (i.e., the actual development and behavior of the disease) will likely only be fully apprehended when we can address the context in which the molecular changes and the disease take place. This threefold description of the nature of cancer most fully describes the vast majority of solid tumors seen in adults (which represents the vast majority of cancer). Accordingly, cancer can be seen as:

(1) A disease of genomic instability.

(2) A disease of altered cellular behavior.

(3) A disease of altered tissue behavior.

The first provides the underlying basis for the development of cancer. The second provides the most traditional view of the requisite acquired characteristics of the cancer cell. The third recognizes that a tumor is not just a collection of cancer cells, but is rather a complex organ or set of organs (primary tumor, metastases) whose properties reflect the interactions between genetically altered cancer cells and the host.

One caveat before exploring this typology: any attempt to organize a description of the nature of cancer should allow us to better think about and to more effectively talk to each other about cancer. One would hope that this organization frames both the types of research questions we ask and meaningfully differentiates the underlying mechanisms. While the former is a matter of convention and utility, the latter is a real and important challenge. The search to understand the nature of cancer leads us to the current consensus of cancer as a complex somatic genetic disease. Any mechanistic typology of cancer should organize a division of characteristics or features about cancer that correlate with the genetic changes that are responsible for those characteristics. The problem is that the more we explore the critical genes and pathways in cancer, the less neatly they seem to sort between the disparate features of nearly any cancer typology. We can think about this as the "one gene-one phenotype" problem of cancer. Cancer emerges as a process whereby new phenotypes become manifest over time. The emergence of these new phenotypes must reflect the accumulated effects of fixed and heritable genomic changes. Yet, the more we look at the function of genes whose activities are altered in this process, the less neatly those functions divide into current descriptions of the distinct characteristics of cancer. Thus, genes associated with control of proliferation of the cancer cell may also be involved in genomic instability or vascularization of tumors. Genes involved in invasion may also be involved in growth control or apoptosis.

One simple explanation for this is that we are not accurately describing phenotype, differentiating between characteristics that are actually distinct manifestations of a single cellular phenomenon. Alternatively, there may be some fundamental principles at work, whereby the distinct attributes of cancer involve molecular pathways that must interact with each other. Despite this complexity, the central challenge of cancer cell biology is to identify the genes whose changes are associated with cancer and to then identify how the changes in those specific genes and gene products are connected to the behavior of the cancer. The simple possibility of cataloging genetic changes and phenotypic changes and then matching them to each other will be hard to achieve. The genotype-phenotype challenge in cancer will more likely require that we address issues such as:

(1) What is the meaning of the particular combination of genetic changes seen in cancer, and how do these changes interact with each other?

(2) Is the order of acquisition of genetic changes important?

(3) How many genetic changes actually contribute to the cancer phenotype?

(4) How does the content of the specific cell lineage affect the pathways and phenotypes of cancer?

(5) How do host factors modify the genotype-phenotype relationships?

Despite the complexity underlying our attempt to correlate cancer genotype with phenotype, great progress is being made in both identifying and characterizing critical "cancer genes" (Hahn et al., 1999). Furthermore, conceptual relationships are emerging that may explain the patterns of genetic changes seen and not seen in cancers. In general, a limited number of pathways, such as Rb and p53, are altered in the vast majority of tumors. A single pathway (i.e., Rb) is altered at only one component of the pathway in each tumor. There are strong and unexplained biases in which pathways and/or the components of a common pathway are altered in particular tumors or tumor types. Finally, tumor development involves phenotypes that drive a cancer cell to acquire a new type of behavior or state, and those that allow the cancer cell to tolerate either the altered state or its consequences.

Therefore, one can view critical genetic events in the development of cancer as series of paired changes. This is not to imply a mechanistic or temporal coupling, but rather the need for a second event to allow the survival of cells altered by a first event. For example, the massive genomic instability associated with the majority of carcinomas (see below) would be expected to result in the activation of checkpoint and protection systems that would lead to cell cycle arrest and/or apoptosis. To become a tumor, a second site (this may actually be multiple "second" sites) must be altered to allow the cell to either ignore the genomic structural abnormalities or to uncouple its recognition from either arrest or apoptosis (for example, by the induction of the p53 pathway). Similarly, there have been reports of alterations in components of a likely mitotic checkpoint (Bub1 and hsMAD1), which might allow cells to survive the anaphase bridges and premature onset of anaphase, that may underlie some aspect of genomic instability in cancer.

Cancer as a disease of genomic instability

It is well documented that cancer is the result of the accumulation of heritable genetic changes in a single clone of cells and its progeny, endowing that clone with the variety of properties we ascribe to cancer. It has been more difficult to demonstrate the

more fundamental question of whether these genetic changes are themselves a manifestation of true genetic instability. The question of whether cancer, which clearly reflects the results of accumulated genetic changes, is a manifestation of an increased rate of production of such changes or rather the clonal expansion and selection of cells having undergone a normal rate of mutation has long been debated. This question has recently been nicely reviewed (Lengauer et al., 1998). However, recent genetic and biochemical evidence strongly supports the notion that genetic instability may be a defining molecular descriptor for most cancers.

There are two types of generally nonoverlapping genetic changes seen in most cancers: subtle sequence alterations and gross changes at the chromosomal and subchromosomal level. The latter are much more common, seen in over 80% of carcinomas, but it is the former that has given us the clearest answer to the critical role of genetic instability in the development of cancer. This is because of the precise identification of inherited genetic changes in well characterized genes involved in either Nucleotide Excision Repair (NER) or Mismatch Repair (MMR) that give rise to cancer. The changes seen in these cancers involve deletions or insertions of small numbers of nucleotides and base substitutions. In most other cancers, the genomic instability is manifested by gross changes observable at the karyotypic level, and include whole chromosome number changes, or, more commonly, sequential losses or duplications, chromosomal translocations, and amplifications and/or deletions of smaller (5–10 Mbase) regions of chromosomes. For all of these manifestations of either subtle or gross instability, assays have now demonstrated that the rate at which the cancer cell accumulates these changes is greatly elevated over that seen in similarly growing cells that lack the particular instability phenotype. It is worth pointing out that the exceptions to this general description of cancer as a "disease of genetic instability" are the cancers, generally noncarcinomas, that possess single, reproducible balanced translocations of specific chromosomal sites (i.e., leukemias, some lymphomas, Ewing's sarcoma). In these cases, there is no evidence of any underlying state of genomic instability.

As highlighted above, genomic instability is associated with either subtle changes (so-called MIN and NIN) or with gross changes (so-called CIN). The most satisfying explanation for this is that a tumor needs to activate one genetic pathway to genetic instability. Such an "either/or" argument actually supports the notion that tumors must "find" one pathway to genetic instability, but once one is acquired, there is no selective advantage to acquire another. While satisfying, we have not formally ruled out the possibility that the simultaneous expression of both MIN and CIN might be selected against (although there is neither evidence nor mechanism for this).

Many questions remain to be answered about genomic instability and cancer. A discussion of three important areas that we need to better understand, including the mechanism of gross instability, the patterns of chromosomal changes in cancer, and allele-specific genomic changes, follows.

Mechanism of gross instability

While the mechanisms underlying MIN (and NIN) are relatively well understood, the mechanisms underlying CIN are not only not understood, they have yet to be cataloged. It is very possible that a large number of totally distinct mechanisms will prove to be responsible, and whether they act by gain-of-function or loss-of-function is unclear. Possibilities include the multitude of genes involved in double-strand breakpoint repair, the many steps involved in appropriate alignment, condensation, cohesion and separation of sister chromatids, centrosome number, and function control, and an increasing number of checkpoint processes that monitor mitotic events. Cancer-associated genes that have been associated with the DNA-damage checkpoint whose loss may be associated with chromosomal segregation abnormalities include ATM, p53, and BRCA1 and 2. Genes associated with abnormal control of centrosomal number and function include p53 and the aurora 2 kinase. Genes associated with a potential spindle checkpoint include BUBl and hMAD2.

Recently, a series of observations, first in the mouse (Chin et al., 1999) and, more recently, in human cancers (Gisselsson et al., 2001), point to an interesting role of shortened telomeres in the generation of many of the genomic features of CIN. Shortened or absent telomeres can lead to the fusion of chromosomes and the formation of dicentric structures. These can form bridges at cell division that are resolved either by fragmentation and/or unequal distribution to daughter cells. The fragmented chromosomes may again fuse and repeat the cycle of breakage-fusion-bridge formation first described by McClintock in 1941. Many aspects of the unstable karyotype of human cancers can be explained via this mechanism (coupled with the capacity, perhaps via loss of p53, to survive these changes). Thus, it may be that the proliferation of cells and the subsequent shortening of telomeres seen in human carcinomas is a critical step in the generation of genomic instability that, in turn, drives the evolution of cancer. While an attractive notion, many questions remain as to the details of such a model. The dependence of this model on telomere length has several implications. It has been proposed as the explanation of why $p53^{+/-}$ mice do not develop common epithelial tumors until they are crossed to a telomerase null background. Mice have much longer telomeres than humans, and the genetically induced shortening of murine telomeres thus "humanizes" the tumor phenotype of the $p53^{+/-}$ mice.

Patterns of chromosomal changes in cancer

If we are to correlate the genomic changes in cancer with meaningful functional changes, we will need to identify, catalog, and interpret those changes. Clear progress has been made in this regard, but it is largely limited, for obvious reasons, to the single, reproducible translocations seen in leukemias, certain lymphomas, and the exceptional nonepithelial solid tumors, particularly those of childhood. We are only now beginning to measure and catalog the changes seen in common human tumors. Which changes are recurrent? Which affect important gene function? How do the observed changes reflect the underlying mechanism of genomic instability? Do the changes only reflect the underlying mechanism, or do they also reflect the selection of changes that confer tumor growth advantage? Other important questions concern the relationship between genomic/genetic changes and tumor development. A large amount of data supports the idea that in tumors with gross genomic instability, the extent of genomic change increases with the temporal development of the tumor. On the other hand, recent data demonstrate that a large amount of gross genomic changes occur early, detectable even in lesions such as DCIS of the breast (Snijders et al., 2001). A recent study of pancreatic cancer (an epithelial tumor) and osteosarcoma (a mesenchymal

tumor) suggests a possible temporal pattern of first telomeric and then interstitial and centromeric breaks as a manifestation of repetitive breakage-fusion-bridge cycles initiated by telomeric shortening.

Allele-specific genomic changes—Where molecular genetics may meet molecular epidemiology

One area of the molecular genetics of genomic instability ripe for exploration is the allelic patterns of the observed chromosomal changes. Tumors may lose or alter up to 50% of their alleles. This is characterized by the common loss of heterozygosity of many loci which is often accompanied by a gain of function of the remaining allele. This gain may result in a normal karyotype of the region but an abnormal allelotype. Often a particular allele is duplicated. Too little is yet known about allele-specific amplifications in cancer. We are at a propitious time now (with the completion of the genome, the identification of large numbers of single nucleotide polymorphisms, and high throughput analysis of tumor samples) to ask whether or not amplifications are random with respect to common human polymorphisms. If tumors select for specific alleles to be lost, retained, gained or amplified, we may be able to identify polymorphisms that either predispose (perhaps weakly) to cancer in the general population or modify the evolution and/or behavior of tumors once begun.

Cancer as a cellular disease of altered circuitry and altered cellular behavior

This is the definition of cancer that has been the most intensively and productively studied over the past several decades. An excellent perspective on this topic was published two years ago in *Cell* by Hanahan and Weinberg (2000), appropriately titled "The Hallmarks of Cancer." They described six acquired capabilities that define the malignant state, four of which I will include in this cellular aspect of the nature of cancer: relative self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, and limitless replicative potential. The challenge that remains, as those authors pointed out, is to cope with both the complexity and the contextual issues that connect what appears to be a limited number of phenotypes and basic molecular circuits with the nature and evolution of each type of human cancer.

Issues of complexity and context come in several flavors. One is the question of the definition of each cancer, and another is how many different cancers there are. Cancers have been traditionally classified by their histogenic origin. This has been refined as the presumed cell of origin has been discerned, first anatomically, then microscopically, and more recently, by the added use of molecular markers. Over the past several decades, we have begun to add molecular markers that are more closely associated with the circuitry and even the molecular pathogenesis of the cancer. These include specific translocations, amplifications, and mutations in molecules relevant to both cell behavior and therapy, such as the estrogen receptor. A recent explosion of comprehensive molecular analyses of cancer, primarily using transcript expression arrays, is revealing new molecular heterogeneity within histogenic classes of cancer (Alizadeh et al., 2000; Perou et al., 2000; Ramaswamy et al., 2001). Despite a rapidly growing literature, it is too early to know what exact picture of tumor heterogeneity will emerge from this approach. Several tentative conclusions from these studies are: (1) that there is considerable heterogeneity of gene expression both between tumor types and, in some cases, within currently classified tumor types; (2) that metastatic lesions appear similar in overall gene expression to their primary tumors; and (3) that heterogeneity may represent some combination of cell of origin, pathogenetic pathways, and sample heterogeneity. Challenges to extracting optimum information from array analysis will include sample preparation, standardized methodologies, fuller and richer annotation of the human transcriptome, and larger collections of data correlated with relevant clinical descriptors.

Another obvious challenge of complexity will emerge as more experimental data fleshes out the circuits and signaling pathways. It is already clear that these pathways themselves are complex, branched, and overlapping. They are, in many cases, beginning to look more like networks that intersect and "crosstalk," rather than simple linear and distinct pathways (Hunter, 1997). While the pathways and networks that we draw define demonstrated connections, it may well be important to refine, annotate, and understand the connectivity maps in terms of the quantitative, temporal, and spatial details of the connections. If we are to understand the flow of information through these networks and how this is perturbed by changes in the proteins involved, we will need new experimental approaches that will add kinetic and other quantitative measures. This will not only be a future experimental challenge, it may also require rethinking the reliability of some of the pathways as we currently perceive them, particularly if the underlying data has relied too heavily on protein overexpression studies. In short, we need to move from a description of cellular circuits as reflecting the possible or potential biochemical connections to a description of the physiological and pathophysiologically relevant connections.

Raising the question of relevance moves us from the challenge of complexity to the challenge of contingency or context. We can consider three types of contingencies within which we need to understand cancer pathways:

(1) How pathways function in the context of the acquired genetic changes in a tumor.

(2) The functioning of pathways in different cell lineages.

(3) The effects of the individual's genetic background.

The pathways implicated in cancer are highly interconnected, but we have little systematic information about cancer cells that allow us to conclude whether or not there are specific or preferred combinations of alterations in cancer cells. It will be interesting and informative to develop a comprehensive database of the multiple altered pathways present in any given tumor to look for such combinatoric genetic changes. This question of which specific alterations tend to occur together in any specific cancer likely intersects with the second contingency issue—that of the effect of the cell of origin of the cancer. An enormous amount of data makes it abundantly clear that different cancers (defined histogenically and by site of origin) display different frequencies of specific genetic changes. For example, APC is lost in the majority of colorectal cancer but rarely in other cancer types, while VHL loss is largely limited to renal cell carcinoma and ras is commonly mutated in pancreatic cancer but rarely in a variety of other tumors. Why is this? We are beginning to understand that the lineage of a cell seems to have a profound effect on how any pathway and/or any component of a pathway is either wired or interpreted. Accordingly, the consequences of changing specific components and specific pathways may be profoundly different in different lineages. These differences may be attributed to the inability of a specific cell type to survive, to compensate, or to "care" about changes in

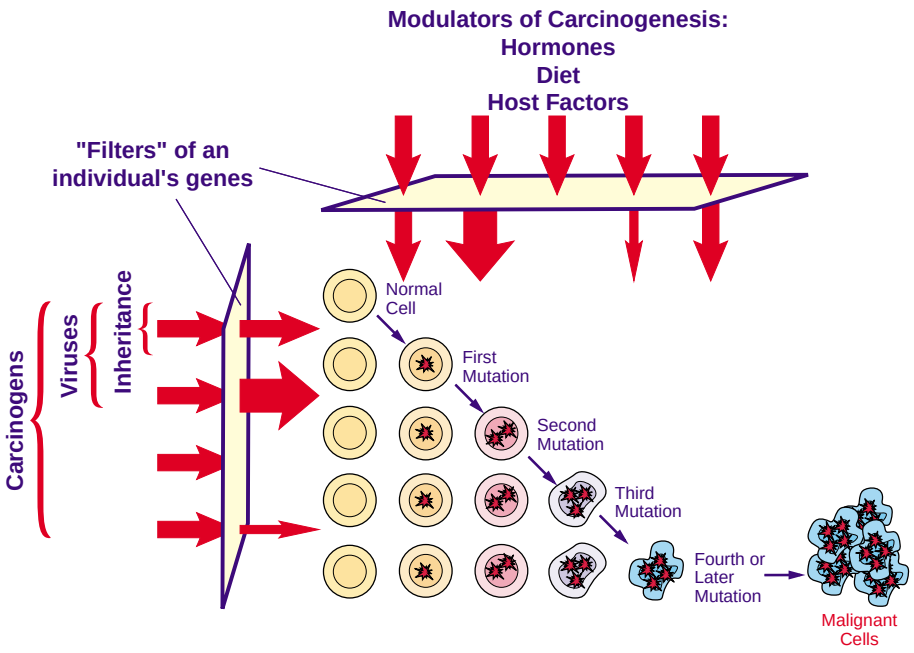

Figure 1. The complex process of carinogenesis

Cancer arises through the accumulation of genetic alterations within a subset of our genes. Certain of these genetic alterations can be hereditary whereas others alterations arise as a result of exposure to carcinogens and certain oncogenic viruses. It is clear that commonality in exposure does not lead to common outcomes in terms of cancer development. Modulators of carcinogenesis include hormones, diet, and a range of other host factors. The impact of exposure to carcinogens as well as the influence of these modulators are in a sense "filtered" by an individual's genes that can eliminate, reduce, or enlarge the impact of a given contributor to the process. In the figure, this modulation by the individual's genes is represented as elimination, reduction, or enlargement of the arrows as they pass through the genetic "filter". Understanding the process of carcinogenesis will require a much more comprehensive understanding of gene-environment interactions than exists today.

any given pathway or component.

One recent study illustrates the type of lineage contingencies that exist and that will explain the molecular peculiarities of specific cancers (Yu et al., 2001). Cycin D1 is overexpressed in up to 50% of human breast cancer. In these tumors, this overexpression is most likely the mechanism by which the Rb pathway is inactivated. When cyclin D1 is homozygously deleted, mice fail to develop mammary tumors when either ras or neu is overexpressed in mammary epithelium. In contrast, mice that overexpress either Wnt1 or myc continue to develop breast tumors in the absence of cyclin D1, Thus, ras and neu, but not these other two oncogenes, must communicate with the Rb cell cycle regulator through cyclin D1. Strikingly, these cyclin D1 deficient mice continue to develop other tumors driven by either ras or neu. In fact, these authors showed that in cyclin D1 null fibroblasts, transformation by ras or neu resulted in elevation of cyclin D2 and cyclin D3, something that mammary epithelial cells could not do. Thus, as the authors point out, the ways these ubiquitous biochemical pathways are wired are different in the various cell lineages.

One of the great challenges in cancer biology, indeed in all human biology, is to understand how distinct genetic variations among individuals effect the manifestation of disease, from environmental exposures to the penetrance and expressivity of either inherited or, in the case of cancer, acquired genetic states (Figure 1). The way that polymorphisms, especially common population polymorphisms, influence the development and manifestations of cancer and even the response to therapy is a challenge that we have barely begun to address. There is every reason to believe that genetic interactions will be an illuminating and important aspect of understanding cancer. Our strongest clues supporting this last statement come from the documentation of clear differences in the expressivity and penetrance of inherited cancer syndromes in humans and from observations about cancer in the mouse. Individuals who inherit mutations in strong cancer susceptibility genes have widely varying probabilities of developing cancer. Some of this may be due to the penetrance of specific mutations, some may be due to environmental and exposure effects, some may reflect stochastic events, and some reflect a rich and powerful effect of the individual's genetic background. Such effects have been abundantly demonstrated in the mouse, where strain-specific effects of expressivity and penetrance are dramatic (Balmain and Nagase, 1998). The effects of these so-called modifier loci can be on almost any aspect of cancer development from initiation to progression (Balmain, 2002). The identification of the specific genes that modify cancer incidence and/or behavior is in its infancy. This process has been hampered by the expense and time that the experiments require, the absence of the complete mouse genome sequence, and the fact that strain-specific effects often reflect the contribution and interaction of multiple polymorphic loci. Despite these difficulties, I believe that the identification of mouse modifier genes, coupled with the development of mouse models of human cancer, will prove to be immensely illuminating. They will provide insight into how critical biochemical pathways interact with the cancer cell circuitry. These modifier pathways may themselves be altered in cancer cells, or may determine how the host interprets and/or responds to primary circuit alterations in cancer. The first and best described murine cancer modifier (Cormier et al., 2000), MOM1, was identified as a strain-dependant variant(s) that modified the expression of intestinal tumors in mice with germline mutations of the APC gene. Interestingly, the gene responsible for at least half of the modifier effect, a secretory phospholipase, is not expressed in the tumor cells, but rather in the normal intestinal crypt cells of the host. The implications of this finding lead us to the third aspect of this tripartite typology of cancer.

Cancer as a disease of dysregulated tissue behavior

Perhaps the greatest challenge facing the cancer biologist will be to move from the discovery, description, and elucidation

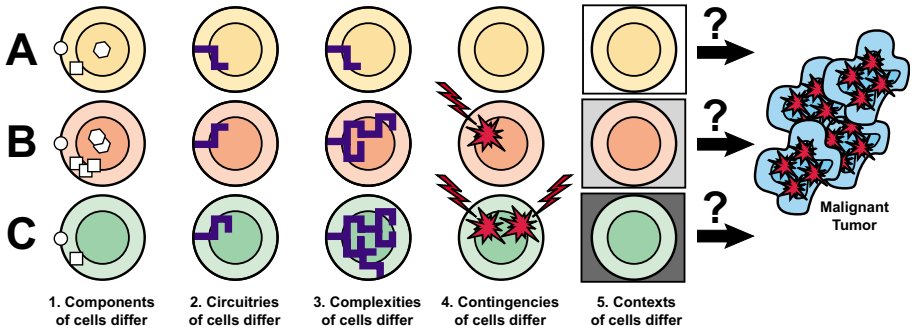

Figure 2. The multiple strands of cancer research

A, **B**, and **C** represent different cancers that are each characterized by: (1)distinctly altered components; (2) distinct alteration of cell circuitries; (3) distinctly altered intracellular signaling networks; (4) the meaning of the altered circuitry is dependent upon the cell lineage and the history of the developing cancer; and (5) the final tumor that results is due to the interactions of the cancer cells in the context the host. In ways that are yet to be elucidated (question marks), all of these phenomena weave together to produce a particular cancer.

of the components and pathways within the cancer cell to studying cancer as the manifestation of a set of complex interactions between the cancer cells and the cells and tissues of the host. This is the least understood area of cancer biology and, from the viewpoint of the patient, the most important. It is in this realm that we must learn to understand how cancer cells subvert the architecture of the tissue in which they develop, create a pathologic organ (called a tumor), spread, and metastasize. While this aspect of cancer biology has largely been focused on the processes of invasion and metastases, it is clear that there are few phenotypes of the cancer cell that do not involve a complex and intimate set of interactions between the cancer cell and the host. Even at the earliest stages of cancer development, the development of dysregulated growth and survival requires an altered set of interactions between the cancer cells and neighboring cells. This may include altered production of paracrine growth control signals (both positive and negative) and altered physical interactions between cancer cells and noncancer cells, as well as between cancer cells and the extracellular matrix (Aplin et al., 1998). Some of the most common alterations in cancer involve changes in the expression of E-cadherin, a cell-cell interaction molecule found on all epithelial cells. Altered patterns of expression of the complex integrin family of proteins are common, resulting in changes that have yet to be deciphered in cancer cell-ECM interactions. These sorts of changes presumably underly many of the aspects of the ability of the cancer cell to grow and survive within the structured confines of a tissue, and are also part of the fact that tumors are not just collections of proliferating cancer cells, as generally studied in the laboratory, but rather are highly reprogrammed tissues made up of cancer cells and conscripted host cells, many of which may be altered by signals from the cancer cells and whose signals may be essential to the cancer cells.

To grow, spread, and metastasize requires that cancer cells achieve a number of goals:

(1) They need to alter the growth inhibitory signals that are normally received from surrounding cells and stroma, either by changing the signals or how they are deciphered by the cancer cells.

(2) They need to lose the normally tight cell-cell and cell-stroma physical interactions via adhesion molecules.

(3) They need to be able to remodel the local architecture, largely through altering the expression and activity of extracellular proteases.

(4) They need to recruit, create, and remodel vasculature (Folkman, 1997). This involves both angiogenesis and lymphangiogenesis (Stacker et al., 2001).

(5) They need to acquire the capabilities of invasion and migration, and spread through tissues and out of the tumor via blood, lymph, or tumor-derived vasculature.

(6) They need to be able to establish new ectopic tumor-tissue structures via the process of metastasis.

There is an emerging body of work adding to this complexity, whereby signals arising in a tumor may reprogram both "normal" and cancer cells. In the former case, normal fibroblasts may be "rewired," coevolving with the cancer cells to support in some essential ways the growth, evolution, and development of the cancer cells (Olumi et al., 1999; Skobe and Fusenig, 1998). The merging sense in both cancer biology and biology in general of the extraordinary plasticity of cells will become an important aspect of understanding the complex tissue biology of a tumor. An intriguing example of this plasticity is the observations that cancer cells may take on the structure and function of vascular cells in a process called vascular mimicry (Hendrix et al., 2001).

In addition, the same one genotype-one phenotype problems discussed earlier in assigning functions to altered cancer cell circuitry arises in attempting to explain the heterotypic biology of tumors. It is problematic and likely wrong to assign a single functional role to a gene product or molecular pathway in a specific cell or tissue phenotype. For example, extracellular proteases, long studied in cancer to explain cancer cell invasion (Werb, 1997), are now recognized to effect angiogenesis (Stetler-Stevenson, 1999) and the control of growth of the cancer cell. Adhesion molecules and their modulation affect cell growth (Aplin et al., 1998), apoptosis, invasion, and metastasis (Johnson, 1991).

Our "parts list" of the complete set of cellular players in any given tumor remains incomplete. We not only will need an enumeration of the cell types engaged in a given tumor, but also a molecular description of the components that define the multiple and reciprocal interactions among all of these cell types.

Finally, metastasis is a particularly critical aspect of tumor cell biology, as it is this process that explains the lethality of cancer. Progress is being made in many aspects of this problem. We are beginning to identify, in certain tumors, what patterns of gene expression correlate with metastatic potential (Clark et al., 2000; Saha et al., 2001). To understand metastasis, we need to explain the multiple ways by which cancer cells leave their site of origin, find their preferred site of ectopic residence, and recreate the tumor tissue at this new site. The ways in which the striking biases of particular organs of metastasis for specific cancers are determined has been the subject of much research and model building. Evidence now supports multiple models

(Liotta, 2001), including matching appropriate growth and growth control factors between the cancer cells and the target organs, the expression of specific adhesion factors that recognize organ-specific molecules found in the vasculature, stroma, or other organ components, and homing mechanisms. The identification of chemokine receptors on specific cancer cells coupled with the organ-specific production of specific chemokines offers a new basis for the role of chemoattractant homing in metastasis (Muller et al., 2001).

Toward a synthesis in cancer biology

While the molecular and cellular components of cancer will continue to be elucidated, the future of cancer biology research will move from a productive reductionism toward a more synthetic approach. This new approach will not ignore reductionist inquiry but will rather incorporate and better annotate the findings gleaned from taking tumors and cancer cells apart. This synthesis of inquiry and approach will require that we consider cancer at various levels and integrate the findings at one level within the context of another (Figure 2). While we continue to identify the components whose functions are central to the behavior of the cancer cell, we will need to characterize these as functioning in a limited number of circuits. These circuits will be more clearly described as highly complex and interacting networks, which in order to be understood will require detailed and quantitative descriptions of how they operate, both temporally and spatially. Next, we will need to document and explain the particularities of what I have referred to as contingencies. This refers to how the cell decodes and responds to alterations in components, circuits, and complex networks as a function of cell lineage, state of differentiation, and the unique molecular history of each cancer. Finally, in order to actually understand the development and behavior of cancer, we will need to understand the context in which the cancer cell develops and survives. This last issue represents, not surprisingly, our greatest challenge, but one that will reveal both the greatest surprises about cancer and the major conceptual breakthroughs of the next decade. The additional challenge of context will involve the elucidation of how multiple allelic variations determine or influence cancer pathogenesis, behavior, and response to intervention within the human population and in any given individual. So, approaches used to gather information at each level will need to be addressed in the context of each of the other levels. Thus, we will need to describe the components, circuits, and complex networks for each relevant contingency and in each cell type of a tumor, as well as the higher order interactions that create new contingencies, etc. This description of the challenge of cancer biology is analogous to the challenge of developmental biology. Indeed, it is not a coincidence that the critical components, pathways, and many of the principles of development are used in and are central to cancer. If we accept the proposed typology of cancer presented here—that it is a disease of dysregulated tissue behavior which, in turn, is a manifestation of the genetically altered behavior of a clonally proliferating and immortal population of cancer cells, which itself is the result of a fundamental disorder of genetic/genomic stability in that clone—then the ultimate synthetic understanding of cancer is very much a problem of a pathologic developmental process.

The use of model systems derived from many species, but most particularly the mouse, needs to be pursued. We will require the further development and maturation of high throughput analysis of genomic, genetic, and epigenetic changes, gene expression, and protein profiles, as well as the corresponding development of new approaches to sample preparation and the ability to assign molecular changes to single cells. The study of complex tumors and imaging techniques that will allow us to study tumor development, growth, spread, and metastases in real time (and that provide structural, anatomic, functional, and molecular information) needs to be developed further. The identification of contributing genetic factors will be achieved by new approaches to the rapid detection of human variation and total genome scan methods, as well as candidate locus and gene examination. In addition, this effort will likely require the use of data derived from mouse genetics and from the examination of recurrent genetic changes and allele-specific gains and losses in the tumor itself. New analytic tools and extensive use of mathematical modeling will be essential if we are going to make sense of the flood and complexity of the data that will be generated by this synthetic approach. The amount of information, the variety of observations, and the combinatoric possibilities indicate that we will need to enhance the acquisition of data and work toward the assembly of new cancer biology databases that will allow us to search for and test the patterns that will yield the synthetic pictures of cancer that we are seeking.

There is little question that the molecular elucidation of cancer is fundamentally changing the way we think about the prevention, detection, diagnosis, and treatment of cancer.

Some few recent clinical advances with both targeted monoclonal antibodies and specific small molecule inhibitors have revealed the potential of translating molecular knowledge to clinical practice, despite all of the remaining unknowns about cancer biology. One can certainly question how much we need to know and understand before we can develop better interventions. The answer is twofold. First, we should and will act opportunistically to attempt to apply what we are learning as that knowledge accumulates. Second, there simply is no definitive answer to the question. My own belief is that we are only at the very beginning of a rational, molecular, and predictable approach to cancer intervention. These new molecular therapies are valuable and exciting. They demonstrate a proof of principle and provide a glimpse into the future. However, the task before us remains daunting. We continue to have very few effective and specific ways to treat the vast majority of common tumors once local therapy is not an option. I hope that this perspective indeed describes that we are at a transition point in cancer biology, one that builds on all of the progress of the last several decades. This is not because we are on the verge of rapidly curing cancer, but rather because we are on the verge of being able to move into a new era of basic cancer research that will begin to weave the disparate and multiple threads of cancer biology together, addressing the enormous complexity of human cancer and preparing us to really apply what we learn to transform the way we intervene to prevent and cure the many diseases we call cancer.

Acknowledgments

I would like to acknowledge Joe Harford for preparation of the figures.

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1 Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20891, USA. klausner@mail.nih.gov. PMID: 12086880. DOI: 10.1016/s1535-6108(02)00020-x. No abstract available. Publication types. Review. MeSH terms. A cancer cell divides at a very fast rate.Cancer cells are unhealthy/bad and donâ€™t help your organs to function.Cancer wouldnâ€™t have been a problem,but these cells donâ€™t die like a normal cell. Cancer doesnâ€™t cause death.Cancer cells donâ€™t help your organs to function,and thatâ€™s why most patients die from organ failuresâ€¦ Cancer cells have damaged DNA,which does not give the action to stop dividing. Cancer just wants to divide,and it wonâ€™t stop untill all your billions of cells are cancerous..!!! 11. The fabric of cancer cell biology-Weaving together the strands. Authors: Richard D Klausner. Cancer Cell 2002 Feb;1(1):3-10. Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20891, USA. View Article and Find Full Text PDF. February 2002. The contribution of VHL substrate binding and HIF1-alpha to the phenotype of VHL loss in renal cell carcinoma. Authors: Jodi K Maranchie James R Vasselli Joseph Riss Juan S Bonifacino W Marston Linehan Richard D Klausner.