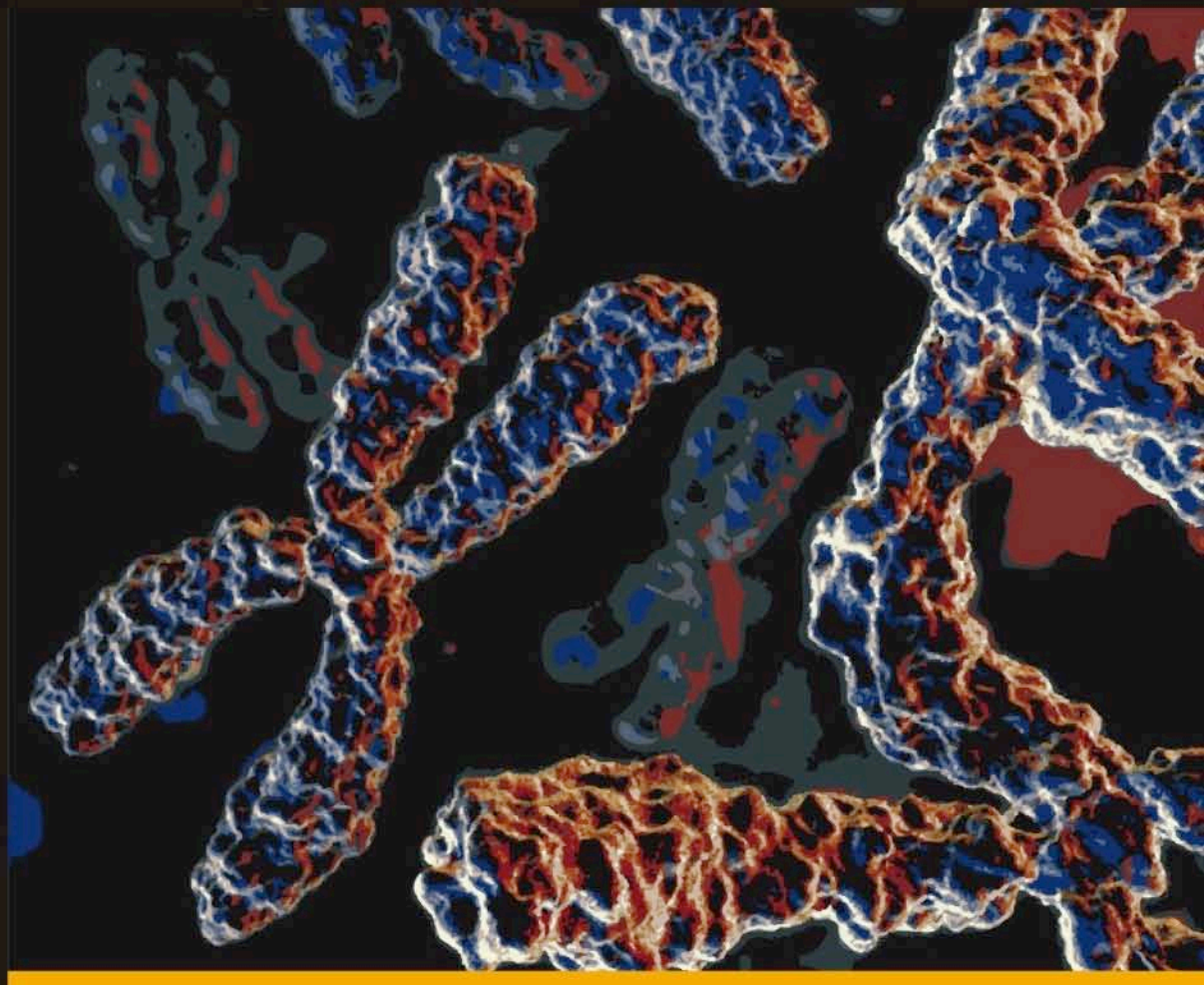
The Chromosomal Imbalance Theory of Cancer

Autocatalyzed Progression of Aneuploidy *is* Carcinogenesis



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Preface

Tumors destroy man in an unique and appalling way, as flesh of his own flesh, which has somehow been rendered proliferative, rampant, predatory, and ungovernable. They are the most concrete and formidable of human maladies, yet despite more than 70 years of experimental study they remain the least understood.

(Rous 1967)

The broadly held conviction among researchers is that cancer ultimately results from an abnormality of the genome. The two principal competing theories on the nature of that abnormality is the subject of this book: Molecular medicine's search for the "material" cause of cancer in the form of gene mutations, and the chromosomal imbalance explanation that cancer results from global alterations in the dynamical relationships among all the genetic and metabolic activities of a cell independent of gene mutations.

In 1969, President Nixon proposed to reduce the budget of the National Cancer Institute (NCI). However, faced with the magnitude of the cancer problem, plus other political considerations, Nixon reversed himself embracing as his own the National Cancer Act sponsored by Senators Kennedy and Rogers and declared a national "war on cancer" in 1971 (Rettig 2006). Planners of this war predicted that technology would conquer cancer as it had conquered space and molecular biology would lead the way.

In 1986, John Bailar and Elaine Smith of the Harvard School of Public Health assessed the overall progress against cancer during the years 1950–1982. In the United States, these years were associated with increases in the number of deaths from cancer, in the crude cancer-related mortality rate, in the age-adjusted mortality

rate, and in both the crude and the age-adjusted incidence rates, whereas reported survival rates (crude and relative) for cancer patients also increased (Bailar and Smith 1986). Notwithstanding progress on minor fronts, they concluded we are losing the war against cancer.

Eleven years later, Bailar and Gornik took another look at how the campaign was going and declared the war against cancer is far from over (Bailar and Gornik 1997). "Will we at some future time do better in the war against cancer?" the authors asked. "The present optimism about new therapeutic approaches rooted in molecular medicine may turn out to be justified, but the arguments are similar in tone and rhetoric to those of decades past about chemotherapy, tumor virology, immunology, and other approaches. In our view, prudence requires a skeptical view of the tacit assumption that marvelous new treatments for cancer are just waiting to be discovered."

In 2004, three federal reports (The CDC's *Morbidity and Mortality Report*, June 25, The Annual Report to the Nation on the Status of Cancer, published in *Cancer*, July 1, and "Living Beyond Cancer: Finding a New Balance" issued by the President's Cancer Panel in early June) said the number of cancer cases in the United States had reached a new high, and more people are alive after a diagnosis of cancer than ever before (Twombly 2004). It was not clear exactly what that declaration meant, however. Some took this to mean there had been marked progress in the treatment of cancer. Others were quick to question the implied widespread treatment success, saying the numbers are inflated by increased detection of non-lethal cancers by screening and there was no information on the quality of life. Even Julia Rowland, director of the NCI's Office of Cancer Survivorship said, "The effect of including those cancers in the data pool is that 5-year survival rates increase because more people who may never have otherwise known they had cancer are now considered survivors, thereby masking the more important question of whether progress has been made in treating advanced solid tumors."

John Bailar, professor emeritus of health studies at the University of Chicago agreed. He pointed out that the reports by the CDC and the President's Cancer Panel directly compared "survival" between two different time frames decades apart. He said that made no sense given the potential for over-diagnosis by increased screening. Even more recently, a 2005 article (Leaf 2004) and two books (Epstein 2005, Faguet 2005) pulled few punches criticizing the paltry progress and dashed hopes in the war on cancer.

In an editorial titled "Our Contribution to the Public Fear of Cancer", Bernard Strauss said, "the scientific community has managed to confuse the public about the causes of cancer and to add to an almost irrational fear of the disease. The only way to allay this fear is to development effective treatment and to understand how cancer develops… . The public's responses to discussions of cancer are reminiscent of societies responses to the threat of epidemics before the nature of infectious disease was understood" (Strauss 1998).

What is the public to make of the confusion caused by the experts themselves? The public's dread of cancer and the fear of plague in the Middle Ages have this in common: no rational explanation for the disease and no way to combat it. But what makes cancer so intractable and mysterious, the biological equivalent of Fermat's last theorem? The answer lies in the way scientists and clinicians have been looking at the problem. Most cancer researchers think they already know the basic cause of cancer: genetic mutations in specific genes (Strauss 1998). However, the gene mutation hypothesis has not led to an understanding of even the most basic questions of how cancer starts and progresses. For example, in a commentary in the *Proceedings of the National Academy of Sciences*, Boland and Ricciardiello asked: "How many mutations does it take to make a tumor?" (Boland and Ricciardiello 1999). The answer was apparently 11,000 (Stoler et al. 1999). Boland and Ricciardiello rightly asked how does this result fit with central concepts such as clonal expansion and multi-step carcinogenesis? Indeed, questions that go to the heart of the mutation theory, which currently says only 4–6 mutations (Hahn and Weinberg 2002b) are needed to cause cancer.

If the current doctrine that cancer is caused by gene mutations was on the right track, the confusion and debate among cancer experts should have diminished in recent years instead of accelerating. Furthermore, cancer statistics should by now show obvious signs of progress but they don't. The worsening situation is leading some cancer researchers to look for an escape from the quagmire of mutation theory. What is needed is a new, more productive way to think about cancer.

The solution one comes up with depends strongly on how one looks at the problem. To see this, consider your favorite puzzle or even better, a well executed magic trick. A world-class magician produces surprise and delight by negating everyday experience and shattering the rules of causality. The *magic* in the magic trick is to make the audience look at the trick in such a way as to make it appear incomprehensible, unfathomable, impenetrable, baffling, perplexing, mystifying, bewildering—how cancer appears today. However, looking at the same magic trick in a different way (the way another magician would) reveals it to be completely consistent with the logic of how things happen. Once the trick is revealed, the magic disappears and the rational world is restored. By looking at the cancer problem in a different way it is possible to lift the shroud concealing the unifying simplicity behind cancer.

Interest in cancer cytogenetics influenced human cytogenetics much more profoundly than is currently appreciated. For example, the main goal behind the study that eventually led to the description of the correct chromosome number in man was to identify what distinguished a cancer cell (Tjio and Levan 1956). The motivation was not primarily an interest in the normal chromosome constitution, which at that time had no obvious implications, but the hope that such knowledge would help answer the basic question of whether chromosome changes lay behind the transformation of a normal cell to cancer (Heim and Mitelman 2009).

Normal human cells turned out to have 23 different chromosomes that come in pairs, half from each parent, to yield a total of 46 chromosomes. Such cells are said to be "diploid." Cells found in solid tumors, on the other hand, typically have 60–90

chromosomes (Shackney et al. 1995a). Their ploidy is "not good," in other words, and the Greek term is "aneuploid." It is a word you will have a hard time finding in the cancer chapters of the leading textbooks of biology.

Recall that the genes (of which there may be 25,000 or so in humans (Collins et al. 2004)) are strung along the chromosomes, so that each chromosome contains thousands of genes. Any cell with a chromosome number different from 46 and not an exact multiple of 23, or with an abnormal complement of chromosomes that add up to 46, is an aneuploid cell. Thus, aneuploid cells contain an imbalance in the complement of genes and chromosomes compared to the normal or "diploid" cell. This imbalance in the chromosomes leads to a wide variety of problems, one of which is cancer.

Another problem caused by aneuploidy that is familiar to most people is Down syndrome. This results when a baby is born with three copies of chromosome 21 instead of the normal two. Just one extra copy of the smallest chromosome, with its thousand or so normal genes, is sufficient to cause the syndrome (Shapiro 1983). Most Down fetuses are spontaneously aborted. Nonetheless, the imbalance is small enough (47 chromosomes) to permit occasional live births. The level of aneuploidy is therefore far below the threshold of 60–90 chromosomes found in invasive cancer, but it gives these patients a head start toward developing the same cancers that normal people get. Down syndrome patients have up to a 30-fold increased risk of leukemia, for example, compared to the general population (Patja et al. 2006, Shen et al. 1995, Zipursky et al. 1994).

There is one important difference between the small chromosome imbalance found in Down syndrome, and the more pronounced aneuploidy of cancer cells. With Down syndrome, the defect occurs in the germ line and so the chromosomal error is present in every cell in the body. But the defect that gives rise to the unbalanced complement of chromosomes in cancer cells is "somatic". That is, it occurs in a particular cell after the body is formed. In the course of life, cells constantly divide by a process called mitosis. When errors in mitosis occur, as they often do, the possibility exists that a daughter cell will be aneuploid.

Aneuploidy destabilizes a dividing cell in much the same way that a dent disrupts the symmetry of a wheel, causing ever-greater distortions with each revolution. As aneuploid cells divide, their genomes become increasingly disorganized to the point where most of these cells stop dividing and many die. But rarely, and disastrously, an aneuploid cell with the right number and combination of chromosomes wins the genetic lottery and keeps right on going. Then it has become a cancer cell.

Cells with a normal number of chromosomes are intrinsically stable and not prone to transformation into cancer. What, therefore, causes normal cells to become aneuploid? That is a hotly contested question. It is known, however, that radioactive particles striking the nucleus or cytoplasm either kill or damage a cell. When the damaged cell then divides by mitosis, an error may arise leading to chromosomal imbalance. In short, radiation can cause aneuploidy. And certain chemicals, such as tars, also give rise to aneuploid cells. Tars and radiation sources are known carcinogens. In fact, all carcinogens that have been examined do cause aneuploidy.

That is a strong argument for the aneuploidy theory of cancer, but in order to understand the controversy one must understand the alternative theory. Everyone has heard of it because it is in the newspapers all the time. It is the gene mutation theory of cancer. According to this theory, certain genes, when they are mutated, turn a normal cell into a cancer cell. This theory has endured since the 1970s, and more than one Nobel Prize has been awarded to researchers who have made claims about it. One prize-winner was the former director of the National Institutes of Health, Harold Varmus. According to some researchers, the mutation of just one, or perhaps several genes, may be sufficient to transform a normal cell into a cancer cell.

In contrast, aneuploidy disrupts the normal balance and interactions of many thousands of genes, because just one chromosome typically contains thousands of genes. And a cancer cell may have several copies of a given chromosome. For this reason alone, aneuploidy is far more devastating to the life of a cell than a small handful of gene mutations.

The fundamental difference between the chromosomal imbalance theory and the reigning gene mutation theory may be put this way. If the whole genome is a biological dictionary, divided into volumes called chromosomes, then the life of a cell is a Shakespearean drama. If one were to misspell a word here and there, in *Hamlet* for example, such "mutations" would be irrelevant to the vast majority of readers, or theater-goers. A multicellular organism is at least as resistant to "gene mutations" as a Shakespeare play.

On the other hand, without "mutating" a single word, one could transform the script of *Hamlet* into a legal document, a love letter, a declaration of independence, or more likely gibberish, by simply shifting and shuffling, copying and deleting numerous individual words, sentences and whole paragraphs. That is the literary equivalent of what aneuploidy does. The most efficient means of rewriting a cell's script is the wholesale shifting and shuffling of the genes, which aneuploidy or chromosomal imbalance accomplishes admirably.

Aneuploidy is known to be an efficient mechanism for altering the properties of cells, and it is also conceded that aneuploid cells are found in virtually all solid tumors. Bert Vogelstein of Johns Hopkins University has said that "at least 90 percent of human cancers are aneuploid." The true figure is 100 percent since there is not one confirmed diploid cancer (Section 4.4.4).

Nonetheless, the presence of mutations in a handful of genes continues to be viewed as a significant, even a causal factor in carcinogenesis, even though any given mutated gene is found in only a minority of cancers. Cells with mutated genes can indeed be found in cancerous as well as normal cells, but it is becoming increasingly clear the vast majority of mutations are innocuous. Hence they are readily accommodated during the expansion of barely viable aneuploid cells as they compete for survival with their more viable chromosomally balanced counterparts. The current emphasis in cancer research on the search for mutant genes in a perpetual background of aneuploidy is a classic example of not seeing the forest for the trees.

Thomas Kuhn remarked that the great theoretical advances of Copernicus, Newton, Lavoisier, and Einstein had less to do with

definitive experiments than with looking at old data from a new perspective. Sufficient (indeed overwhelming) evidence is already at hand to convict aneuploidy of the crime of cancer and release gene mutations from custody (Aldaz et al. 1987, Aldaz et al. 1988a, Aldaz et al. 1988b, Brinkley and Goepfert 1998, Duesberg et al. 1998, Duesberg 1999, Duesberg et al. 2000a, Duesberg et al. 2000b, Duesberg et al. 2000c, Duesberg et al. 2001a, Duesberg et al. 2001b, Duesberg and Li 2003, Duesberg 2003, Duesberg et al. 2004a, Duesberg et al. 2004b, Duesberg et al. 2006, Fabarius et al. 2003, Heng et al. 2006b, Klein et al. 2010, Li et al. 1997, Li et al. 2000, Li et al. 2009, Liu et al. 1998, Rasnick and Duesberg 1999, Rasnick and Duesberg 2000, Rasnick 2000, Reisman et al. 1964a, Reisman et al. 1964b, Ye et al. 2009). Nevertheless, the gene mutation theorists, when faced with the undeniable evidence that aneuploidy is necessary for cancer, have adopted a fall-back position. They argue that gene mutations must initiate the aneuploidy (Sen 2000), or as the *Scientific American* reported, referring to a researcher in Vogelstein's lab, "[Christoph] Lengauer insists aneuploidy must be a consequence of gene mutations" (Gibbs 2001). There would be no need to "insist" if there were proof that gene mutations really do cause aneuploidy and cancer.

What would gravely weaken the aneuploidy theory would be confirmed cases of diploid cancer (in which the tumor cells have balanced chromosomes), and with the culprit genes found lurking in every cell. That would go a long way toward proving the gene mutation theory. But where has that been demonstrated? It would be a front-page story. The truth is that researchers have not yet produced any convincing examples of diploid cancer.

In fact, the evidence is going the other way. There is a growing list of carcinogens that do not mutate genes at all (Section 4.1.4). In addition, there are no cancer-specific gene mutations (Section 4.4.2). Even tumors of a single organ rarely have uniform genetic alterations (Section 4.4.3). And, in a rebuttal that should be decisive, no genes have yet been isolated from cancers that can transform normal human or animal cells into cancer cells (Section 4.4.4). Moreover, the latent periods between the application of a carcinogen and the appearance of cancer are exceedingly long,

ranging from many months to decades, in contrast the effects of mutation are instantaneous (Section 4.4.5).

The goal of billions of dollars and decades of research was to come up with a clear and simple statement of how cancer genes cause or promote cancer. This was certainly the hope and expectation of most cancer researchers. One of the hallmarks of a bad theory is when its evolution becomes so complex and confused that experts in the field have difficulty explaining it. Thomas Ried, a major researcher at the National Cancer Institute in Bethesday, recently labored to…

> *speculate that the activation of specific oncogenes, and the inactivation of tumor suppressor genes act in concert with the deregulation of genes as a consequence of low-level copy number changes that provide the metabolic infrastructure for increased proliferation. One of the challenges in understanding the genome mutations in carcinomas will be to elucidate whether the presence of a tumor suppressor gene on frequently lost chromosomes, or the presence of an oncogene on frequently gained chromosomes is sufficient to fully explain the reason for the defining and recurrent patterns of genomic imbalances. In other words, we will need means to experimentally dissect the relative contribution of specific oncogene activation vis-a-vis the global transcriptional deregulation imposed by chromosome-wide copy number changes. Only then will we be in a position to truly verify or falsify Boveri's central statement, i.e., the dominant role of inhibiting and promoting chromosomes that formed the basis for his chromosome theory of cancer.*
>
> (Ried 2009)

The conceptual barriers to accepting aneuploidy as the cause of cancer are not trivial but they shrink in comparison with the political and sociological obstacles. US taxpayers have forked over hundreds of billions of dollars in the war on cancer only to find that after 40 years of battling viruses, "oncogenes", and "tumor suppressor" genes we are losing the war (Epstein 1998). But it is a one-front war with almost no resources devoted to alternative approaches. In spite of a century of evidence implicating aneuploidy as the cause of cancer, a leading researcher guesses that, "If you were to poll researchers … 95 percent would say that the accumulation of mutations [to key genes] causes cancer" (Gibbs 2001).

The biotech industry has bet heavily on cancer diagnostics and therapeutics based entirely on the gene mutation theory. The highly publicized sequencing of the human genome, the commercialization of diagnostic tests for cancer genes (Arnold 2001, Hanna et al. 2001, Wagner et al. 2000), and the hype about Gleevec being "at the forefront of a new wave of cancer treatments [that] differs from other existing chemotherapies because it affects a protein that directly causes cancer" (McCormick 2001) make it even more difficult for researchers to consider the possibility that mutant genes may not cause cancer after all.

Max Planck said that, "A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it" (Planck 1949). It is encouraging to see that a new generation of cancer researchers are more inclined to accept aneuploidy as an alternative to gene mutation.

Chromosomal imbalance theory shows how gene mutations are not powerful enough to cause cancer (Section 5.4). It explains how cancer is initiated (Chapter 5) and why progression takes years to decades (Section 6.1.3). It explains the global or macroscopic characteristics that readily identify cancer: anaplasia, autonomous growth, metastasis, abnormal cell morphology, DNA indices ranging from 0.5 to over 2, genetic instability, and the high levels of membrane-bound and secreted proteins responsible for invasiveness and loss of contact inhibition (Chapters 5 & 6). It explains the common failure of chemotherapy (Section 7.3) and why cancer cells often become drug resistant even to drugs they were never exposed (Sections 5.3.5 & 6.2.4). It provides objective, quantitative measures for the detection of cancer and monitoring its progression (Section 7.2). It suggests non-toxic strategies of cancer therapy and prevention (Section 7.3). The chromosomal imbalance theory is the most comprehensive, productive, and satisfying explanation of carcinogenesis. In short: *The Autocatalyzed Progression of Aneuploidy is Carcinogenesis.*

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