

The aneuploidy theory of cancer and the barriers to its acceptance

David Rasnick, PhD

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Introduction

Normal human cells have 23 different chromosomes that come in pairs. They yield a total of 46 chromosomes. Such cells are said to be “diploid.” Cells found in solid tumors, on the other hand, typically have between 60 to 90 chromosomes (1). Their ploidy is “not good,” in other words, and the Greek version of that is “aneuploid.” It is a word that you will have a hard time finding in the cancer textbooks.

Recall that the genes (of which there may be 40,000 or so in humans) are strung along the chromosomes, so that each chromosome contains thousands of genes. Any cell with a chromosome number different from 46, 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 is Down’s 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 (2). Most Down’s 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’s Syndrome patients have up to a 30-fold increased risk of leukemia, for example, compared to the general population (3, 4).

There is one important difference between the small aneuploidy found in Down’s Syndrome, and the more pronounced aneuploidy of cancer cells. With Down’s, 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 cell in much the same way that a dent disrupts the symmetry of a wheel. It leads to 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 die. But rarely, and disastrously, an aneuploid cell with the right number and combination of extra 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 if radioactive particles strike the nucleus of a cell, chromosomes can be shattered. When that damaged cell then divides by mitosis, an error may arise. Chromosomal imbalance may then result. 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 so far do cause aneuploidy.

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

The fundamental difference between the aneuploidy 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 may be 100 percent. For references supporting the claim that cancers are invariably aneuploid see Li et al. 2000 (5).

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 the most likely reason is that they 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 in hand to convict aneuploidy of the crime of cancer and release gene mutations from custody (5-16). 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, (17) 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" (18).

There would be no need for him to "insist" if there were proof that gene mutations really do cause 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. In addition, there are no cancer-specific gene mutations. Even tumors of a single organ rarely have uniform genetic alterations. 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. Furthermore, 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.

If the medical profession and biotechnology industries were to embrace the aneuploidy theory of cancer, cancer research and the flood of new technologies would at last become biologically and clinically relevant. There are, however, two formidable barriers to the ascendance of the aneuploidy theory of cancer: the first is conceptual; the second is political and sociological.

Overcoming the Conceptual Barriers

Boveri's Aneuploidy theory of cancer

The aneuploidy theory was introduced by David von Hansemann in 1890 (19) and first formally stated by Theodor Boveri in 1914 (20). Almost the first thing that researchers noticed when they looked at cancer cells under the microscope was that they had excess chromosomes.

Aneuploidy provides a simple and coherent explanation for all the properties of cancer (9-16). But precisely because the aneuploidy theory was proposed so long ago, scientists today are inclined to think (if they know about Boveri at all) that some fundamental flaw in the theory must have been discovered. They also assume that the gene mutation theory of cancer must be superior because it is newer and uses the latest sexy technologies.

Some American researchers, eager to dismiss the aneuploidy theory, ask, “What is the mechanism?” They remind Athel Cornish-Bowden (21) of the obstinate rejection of Alfred Wignener’s theory of Continental drift by American geologists (22) on the grounds that he could offer no mechanism for how the continents moved. In 1914, Boveri offered the first coherent explanation (including a mechanism) of how chromosomal imbalance leads to cancer (20). The developmental consequences of chromosomal imbalance in sea-urchin eggs suggested to him that malignant tumors could be due to an abnormal chromosome constitution originating during cell division.

The only other author with similar ideas was von Hansemann (19). He captured the essence of cancer as, “a process carrying the cell to some entirely new direction—a direction, moreover, which is not the same in all tumors, nor even constant in the same tumor.... The [cancer] cell then is one in which, through some unknown agency, a progressive disorganization...occurs, which in turn results in...a new biologic entity, differing from any cell present at any time in normal [development].” (Translated by Whitman (23)). Hansemann’s “unknown agency” is the relentless randomization of the genome caused by aneuploidy.

Boveri extended Hansemann’s insight. The essence of Boveri’s hypothesis is that cancer results from “a certain abnormal [chromosome] constitution, the way in which it originates having no significance. Each process which brings about this constitution would result in the origin of a malignant tumor” (20). His theory predicts that cancer results from a *single* cell that has acquired an abnormal chromosome constitution. In other words, he predicted the clonal origin of cancer.

It is well known that a tumor cell has an abnormal metabolism. According to Boveri, “if the individual chromosomes have different qualities, chromosome aberrations will result in deviant metabolic functions. If, therefore, certain chromosomes are missing and others are present in abundance, certain substances will be produced also in abundance, and there will be a deficiency in others.” (24)

In Boveri’s time X-rays and certain chemicals were known to cause chromosomal imbalance. Boveri said the time interval between the time of the insult and the origin of a tumor may be explained by the assumption that the cancer-causing agent first interferes with the process of cell division, producing an aneuploid cell. In the second step, the aneuploid cell must be stimulated to divide further, producing daughter aneuploid cells. In heavily proliferating tissues, the risk of a tumor is increased.

Boveri points out that a natural consequence of his aneuploidy theory is that the risk of tumors would increase with age since in aging cells the process of cell division is more frequently disturbed (24). (In addition, enough time has elapsed in an older organism for many cell

divisions to have occurred.) Boveri even predicted tumors that had the correct number of chromosomes but with an abnormal complement—the so-called pseudodiploid cancers. Boveri’s aneuploidy theory of cancer is as valid today as it was in 1914.

Metabolic control analysis supports the aneuploidy theory of cancer

In November, 1996, Peter Duesberg left for the first of many trips to Mannheim to work on aneuploidy as a possible cause of cancer. I stayed at Berkeley and studied the literature on aneuploidy and the consequences of changes in gene dose. One day I came across Charles Epstein’s book *The consequences of chromosome imbalance: principles, mechanisms, and models* (25). When I happened upon a figure extracted from a paper by Henrik Kacser and James Burns, it changed my life. I immediately realized that the reigning gene mutation hypothesis of cancer was almost certainly wrong and that the aneuploidy theory of cancer was almost certainly right.

In 1973 Kacser and Burns (26), and independently Heinrich and Rapoport (27), invented the field of metabolic control analysis. It is a quantifiable means of analyzing changes in a cell, tissue, or organ by taking into consideration the combined activities of all the metabolic elements (all the gene products) that contribute to the phenotype (stable characteristics) of the whole. For systems as complex as a cell, changes in the activities of a few or even scores of specific genes would be buffered by the many thousands of other genes contributing to the overall properties of a cell. There was simply no way for a handful of “oncogenes” or “tumor suppressor” genes to perturb a normal cell sufficiently to turn it into a massively abnormal cell.

At UC Berkeley we have shown that transforming the robust normal cell into a cancer cell requires massive changes in the number and composition of chromosomes (14). Aneuploidy provides the necessary boost in genetic material leading to cancer. It is entirely independent of gene mutation.

The effect of aneuploidy on cells can be visualized by analogy with an automobile factory, in which each assembly line corresponds to a chromosome. An “aneuploid” assembly line would randomize the output of an automobile factory and produce cars with five wheels, three brakes, two engines, no transmission, etc., and every car would be different from the one before. Most such cars wouldn’t function, and would go directly to the junkyard. By chance, however, the aneuploid factory would also produce the rare, bizarre car that worked well enough to appear on the highways and keep right on running when you slammed on the brakes! It would be a menace to the society of normal cars.

In this analogy, the genes correspond to individual workers on the assembly lines. The effect of “mutating” individual workers is much more limited than randomly altering the number and composition of the assembly lines. Workers typically work at a fraction of their capacity. If the output of a few individual workers in an assembly line was “mutated” by sickness, death or vacation, the effects would be buffered by the remaining un-mutated workers upstream and downstream and by the redundant capacity built in to the workforce. The overall output and quality of cars would not noticeably change. By the same token, alterations in a handful of

specific genes (28, 29) are insufficient and probably irrelevant to the generation of cancer because their numbers are too few to alter the normal cell.

The attraction of the gene mutation theory of cancer was its promise of simplicity: cancer resulted from a manageable number of specific mutations. A manageable number was the hoped-for key to unlocking the mysteries of cancer and to the taming of an ever growing modern scourge (30). Instead, we find that the seven mutations proposed to cause colon cancer (31) are drowned in an aneuploid sea of nearly 5,000 additional genes in the aneuploid cells of a cancerous colon (32).

Far from providing insights into the nature of cancer, and hence into prevention and more effective treatments, the gene mutation theory is now so burdened with the complexity of its details that it has lost all explanatory power. Analyzing close to 49,000 genes of normal and cancer cells (colon and pancreas), Zhang et al. acknowledged that, “most of the genes could not have been predicted to be differentially expressed in cancers” (32).

Results such as these will eventually kill what Stephen Friend, CEO of Rosetta Inpharmatics in Seattle, calls the “my-favorite-gene approach.” He adds: “God, were we stupid!” (33).

Political & Sociological Barriers

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 tens of billions of dollars in the war on cancer only to find that after 20 years of battling viruses, “oncogenes”, and “tumor suppressor” genes we are losing the war (30). 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” (18). I think it is a safe bet that if 50 percent of cancer-research funds went towards investigating the role aneuploidy plays in cancer, a poll of researchers would soon show that close to half would say that chromosomal imbalance causes cancer. Scientists, these days, tend to accept or reject a theory depending on whether or not there is funding for it.

Our lab at the University of California at Berkeley has been unable to get a penny from the National Institutes of Health or the National Cancer Institute to investigate aneuploidy and cancer. Our work is supported entirely by private benefactors and volunteer help. Peter Duesberg is forced to spend part of each year in Germany because there is some funding in Mannheim for work on aneuploidy.

With so many careers and reputations dependent on the failed gene mutation theory, researchers cannot afford to question something that has supported them for decades. The highly publicized sequencing of the human genome, the commercialization of diagnostic tests for cancer genes (34-36), and the recent 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” (37) make it even more difficult for researchers to consider the possibility that mutant genes may not cause cancer after all.

It would not help the images of the cancer research establishment and the multi-billion dollar biotech industry if it became widely known that an unfunded lab just may have come up with a preferable explanation of the cause and progress of cancer. If a small group with virtually no money has rediscovered the cause, why should taxpayers continue to dole out billions of dollars for work on mutant genes that has never panned out? And what would happen to the biotech industry that has bet so heavily on cancer diagnostics and therapeutics based entirely on the gene mutation theory?

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” (38). We will soon see if he is right, for the old guard of mutant gene researchers is indeed getting old. It is encouraging to see that a new generation of cancer researchers are more inclined to consider aneuploidy as an alternative to gene mutation.

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