## APPENDIX-D

### Radiation-Induced Genomic Instability: What It Is and Why It Is Important



### • Part 1. Genomic Instability: "One of the Hallmarks of the Cancer Cell"

Genomic instability --- also called "genetic instability" and "chromosomal instability" --- refers to abnormally high rates (possibly accelerating rates) of genetic change occurring serially and spontaneously in cell-populations, as they descend from the same ancestral cell. Some additional terms are discussed at the end of this Appendix, Box 1.

By contrast, normal cells maintain genomic STABILITY by operation of elaborate systems which ensure accurate duplication and distribution of DNA to progeny-cells (Cheng 1993, p.124), and which prevent duplication of genetically abnormal cells. These systems ("metabolic pathways") involve an estimated 100 genes (Cheng 1993, p.142).

Why is genomic instability so important? Many (not all) cancer biologists now believe that genomic instability "not only initiates carcinogenesis, but also allows the tumor cell to become metastatic and evade drug toxicity" (Tlsty 1993, p.645), and "The loss of stability of the genome is becoming accepted as one of the most important aspects of carcinogenesis" (Morgan 1996, p.247), and "One of the hallmarks of the cancer cell is the inherent instability of its genome" (Morgan 1996, p.254).

Although such observations are far from new (Part 2), they certainly did not receive the attention which they merit until recently.

### • Part 2. A Deep Insight from 1914, Slowly Confirmed

It was the year 1956 when the normal number of human chromosomes per cell was firmly established as 46. Soon thereafter, it became clear that cells of advanced Cancers have often evolved an abnormal number of chromosomes ("aneuploidy").

#### 1914: Theodor Boveri's Great Insight

Such observations were consistent with the prediction of Theodor Boveri (Boveri 1914), a great German embryologist who postulated that malignancy is the result of inappropriate balance of instructions (genetic information) in the tumor cells. Such "imbalance" can result not only from numerical chromosome aberrations, but also from structural alterations within the 46 chromosomes. As a leading cause of structural chromosome aberrations (deletions, acentric fragments, translocations, inversions, dicentrics, etc.), ionizing radiation is well-established.

When my colleagues and I initiated a research program in 1963 (at the Atomic Energy Commission's Livermore National Laboratory), to test Boveri's hypothesis, there was very little interest in the concept. Although the techniques for detecting structural chromosome aberrations were extremely crude then, compared with current techniques, we were making gradual progress (Minkler 1970, + Minkler 1971). However, the Atomic Energy Commission became angry with me after a paper I presented at an IEEE Symposium (Gofman 1969-b), and canceled our funding in the early 1970s (Seaborg 1993, Chapter 8, "Challenge from Within," + Terkel 1995, pp.406-408).

1976: Peter C. Nowell's Classic Paper on Tumor Evolution

In October 1976, the journal Science published Peter C. Nowell's classic paper entitled, "The Clonal Evolution of Tumor Cell Populations" --- a paper almost always cited by today's analysts of genomic instability. Among other things, Nowell's 1976 paper discussed evidence, from various analysts, indicating that as tumor cells become increasingly aneuploid, the malignancy becomes increasingly aggressive (Nowell, p.25). Reasoning from the available evidence at that time, Nowell proposed the following model of multi-step carcinogenesis:

Tumor initiation occurs by an induced change in a single, previously normal cell, which makes the cell "neoplastic" (partially liberated from normal growth controls) and provides the cell with a selective growth advantage over adjacent normal cells (Nowell, p.23).

"From time to time, as a result of genetic instability in the expanding tumor population, mutant cells are produced ... Nearly all of these variants are eliminated, because of metabolic disadvantage or immunologic destruction ... but occasionally one has an additional selective advantage with respect to the original tumor cells as well as normal cells, and this mutant becomes the precursor of a new predominant subpopulation" (Nowell, p.23). And:

"Over time, there is sequential selection by an evolutionary process of sub-lines which are increasingly abnormal, both genetically and biologically ... Ultimately, the fully developed malignancy as it appears clinically has a unique, aneuploid karyotype associated with aberrant metabolic behavior and specific antigenic properties, and it also has the capability of continued variation as long as the tumor persists" (Nowell, p.23). And:

"The major contention of this article is that the biological events recognized in tumor progression represent (i) the effects of acquired genetic instability in the neoplastic cells, and (ii) the sequential selection of variant subpopulations produced as a result of that genetic instability" (Nowell, p.25).

The recent surge of interest in genomic instability reflects the recognition that the cancer process represents a trip (or set of trips) from the stable genome to the genome with diverse deviations. It has been a long wait for Boveri.

## • Part 3. Ionizing Radiation (Xrays Included): A Cause of Genomic Instability

Today, laboratory researchers are performing reality-checks on this logic: Genomic instability can be initiated and intensified by any type of genetic mutation (including chromosome aberrations), when such mutation alters some of the DNA which maintains genomic STABILITY. Of course, such DNA includes the numerous DNA segments which govern DNA synthesis, cell-division, and also the routine REPAIR of the genome --- the "repair genes" (Cheng 1993, p.131; Morgan 1996, p.248).

When a mutagen has induced genomic instability in a cell, some of the cell's descendants will experience new and unrepaired genetic abnormalities at an excessive rate, even though the descendants themselves received no exposure to the mutagen used in the experiment. This occurs because such cells have inherited a genome which was injured with respect to maintaining genomic STABILITY.

Aneuploidy, Deletions, Gene-Amplifications

Very recently, a technique has been developed for efficiently detecting three of the types of chromosome aberrations which are very prominent in genomic instability: Aneuploidy (wrong number of chromosomes), deletions (permanent removal of DNA segments, long or short), and gene-amplifications (extra copies of specific DNA segments). This technique, called Comparative Genomic Hybridization, was first described by Kallioniemi (1992, in Science). However, such a technique does not detect many other kinds of mutations.

The nature of the genetic code is such that mutations need not be gross in order to have gross biological consequences. For instance, permanent removal of a single nucleotide (a micro-deletion) can totally garble much of a gene's code, by causing what is called a "frame-shift." Then this non-functional gene can be the phenomenon which wrecks part of the system which would otherwise maintain genetic STABILITY.

Amplification (instead of injury), of the crucial genes in the stability-system, also can permit a cell to escape the controls which otherwise prevent duplication of cells with injured genomes. Evidence is developing that gene amplification is associated with dicentric chromosomes and circular acentric fragments called "double minutes" (DiLeonardo 1993, p.656) --- very well-known products among the consequences of ionizing radiation.

The sequence, in which various mutations accumulate in tumor cells, may or may not matter. "For example, one or more pre-cancerous mutations might lie dormant until additional mutations create an environment in which the prior changes confer a selective advantage" (DiLeonardo 1993, p.655, citing Kemp 1993, + Fearon 1990, + Temin 1988).

Confirmations: Ionizing Radiation Is a Cause of Genomic Instability

The fact, that ionizing radiation is a mutagen capable of causing all known types of genetic mutation --- from micro to gross, at any DNA location along any chromosome --- made it utterly predictable that ionizing radiation would be a cause of genomic instability. Indeed, one of the last projects completed by our research group at the Livermore Laboratory, before the Atomic Energy Commission shut down our work, was a demonstration which showed that ionizing radiation can induce genomic instability. Our experiments used gamma rays and cultured human fibroblasts (Minkler 1971).

During recent years, multiple experiments have confirmed the fact that ionizing radiation can cause genomic instability. Such results have been observed after both low-LET radiation (such as xrays and gamma rays) and high-LET radiation (such as alpha particles). Among numerous papers, see, for instance:

Kadhim 1992;
Holmberg 1993 (who cites Minkler 1971);
Marder 1993 (especially p.6674);
Mendonca 1993;
Kadhim 1994;
Kronenberg 1994 (radiation dose-response, p.605);
Kadhim 1995;
Morgan 1996 (review).

No Discontinuity between Cause and Effect

In the mass media, some writers have expressed astonishment that radiation-induced genomic instability is not detected until several cell-divisions have occurred after the radiation exposure. They seem to imagine that the delay reflects a mysterious discontinuity between cause and effect. There is NO discontinuity, of course --- a point made explicitly in Kadhim 1992 (p.739). With current techniques, and with uncertainties about where to search closely among a billion nucleotides, it is just not possible to detect every intermediate step.

## • Part 4. Implications: Curing vs. Preventing Cancer

The induction of genomic instability in a cell does not guarantee that it will become malignant. Genomic instability increases the RATE of mutation in that cell and its descendants, and with this higher rate, the cells each have a higher PROBABILITY that at least one of them will accumulate all the genetic powers of a killer-cancer. These powers include the ability to thrive BETTER than normal cells, to invade inappropriate tissue, to adapt to the new conditions there, to recruit a blood supply, to fool the immune system, and many other properties.

No one claims, yet, that genomic instability must precede every case of Cancer. However, genomic instability helps to explain why Cancer is sometimes called "at least a hundred different diseases." Indeed, genomic instability means that each case of Cancer may develop a genome like no other case. Is it any wonder that individual tumors often differ in behavior from each other?

Nowell's 1976 paper was certainly not the last one to observe that Cancers become increasingly deviant in their genomes, as they "advance." Tlsty 1993 (p.645) cites several more recent papers. Near the end of his paper, Nowell wrote (p.27):

"The fact that most human malignancies are aneuploid and individual in their cytogenetic alterations is somewhat discouraging with respect to therapeutic considerations ... With variants being continually produced, and even increasing in frequency with tumor progression, the neoplasm possesses a marked capacity for generating mutant sub-lines, resistant to whatever therapeutic modality the physician introduces ... The same capacity for variation and selection which permitted the evolution of a malignant population [of cells] from the original aberrant cell, also provides the opportunity for the tumor to adapt successfully to the inimical environment of therapy, to the detriment of the patient."

And Some Lessons:

(A) • Recognition, that genomic instability constitutes a serious obstacle to curing Cancer, has stimulated strategies to evade the problem --- perhaps by preventing a tumor from acquiring its necessary blood-supply. For some 30 years now, Judah Folkman has been an admirable pioneer in researching this aspect of angiogenesis.

(B) • The quickest path to less cancer-misery in the future would be a policy of reducing exposure to carcinogens.

(C) • Ionizing radiation is almost certainly the most potent carcinogen to which vast numbers of people are actually exposed (see Part 5).

**• Part 5. Five Key Facts and Three Moderate Comments**

(1) • Ionizing radiation is a mutagen having special properties which make some radiation-induced genetic injuries complex and impossible for a cell to repair correctly --- quite unlike the routine damage from endogenous free radicals (Appendix-B, Part 3a, + Appendix-C).

(2) • Ionizing radiation is a mutagen which undeniably can cause every known kind of mutation, at any DNA location along any chromosome. The body does not always eliminate cells having harmful mutations. If it did, there would be very little Cancer --- and no inherited afflictions.

(3) • Ionizing radiation is a mutagen known to induce genomic instability (Parts 2 and 3, above).

(4) • Ionizing radiation is a human carcinogen at every dose-level, not just at high doses; there is no threshold dose. A single photon or a single high-speed particle can result in unrepairable genetic damage (Appendix-B).

(5) • Ionizing radiation is a mutagen observed to induce virtually every kind of human Cancer (Chapter 2, Part 4c).

And the Comments:

(1) • In view of all the five facts above, it would be inappropriate to doubt the health-menace of low-dose ionizing radiation.

(2) • And in view of all the five facts, it is strange --- in studies which attempt to explain a difference in cancer-rates between two groups --- that the question is so seldom asked: How do the radiation histories differ between the groups? In view of the five facts above, it should be the FIRST question.

(3) • And in view of the five facts, it might be appropriate for the American Medical Association, the National Cancer Institute, the American Cancer Society, the American College of Radiology, the American Society of Radiologic Technologists, and dozens of similar organizations, to insist that uselessly high exposures to ionizing radiation, in pre-cancer medical procedures, be eliminated. The ways are known (Chapters 1 and 2).

Today, the two largest sources of voluntary radiation exposure are (i) pre-cancer medical procedures, including CT scans and fluoroscopy (NCRP 1987, p.59, + NCRP 1989, p.69) and (ii) cigarette smoking --- which delivers appreciable alpha-particle radiation to the lungs (Chapter 48, Part 1c). As for involuntary exposures accumulated from nuclear pollution, they have been poorly ascertained --- to put it in a kindly fashion.

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Box 1 of Appendix-D

Mini-Glossary

• GENOME. A person's genome is one set of his (or her) genes. The human genes, which control a cell's structure, operation, and division, are located in the cell's nucleus. The full human genome (estimated at 50,000 to 100,000 genes) is present in every cell-nucleus, even though many genes are inactive in cells which have specialized functions (the "differentiated" cells).

• GENES AND CHROMOSOMES. Genes are composed of segments of DNA. In normal cell-nuclei, the DNA is distributed among 46 chromosomes (23 inherited at conception from a person's father, and 23 from the mother). Each chromosome consists of one very long strand of DNA and numerous proteins, which are required for successful management of the long DNA molecule. The longest chromosomes each "carry" thousands of genes. Every time a cell divides, the cell must duplicate the 46 chromosomes and must distribute one copy of each to the two resulting cells.

• THE CODE. The DNA of each chromosome is composed of units --- "nucleotides" of four different types (A, T, G, C). These nucleotides are linked to each other in linear fashion. The sequence of the four types of nucleotides is critical, because the sequence produces the "code" which (a) determines the function of each particular gene, (b) identifies the gene's start-point and stop-point along the DNA strand, and (c) permits certain regulatory functions. The code of the human genome consists of more than a billion nucleotides.

• THE MITOCHONDRIAL DNA (mtDNA). Outside the nucleus, human cells also have some "foreign" DNA located in structures called the mitochondria. This small and separate set of DNA does not participate in the 46 human chromosomes, and is not part of "the genomic DNA." The mitochondria are inherited from the mother.