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What Can Chemical Carcinogenesis Shed Light on the LNT Hypothesis in Radiation Carcinogenesis?

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Abstract

To protect the public's health from exposure to physical, chemical, and microbiological agents, it is important that any policy be based on rigorous scientifically based research. The concept of "linear no-threshold" (LNT) has been implemented to provide guideline exposures to these agents. The practical limitation to testing this hypothesis is to provide sufficient samples for experimental or epidemiological studies. While there is no universally accepted understanding of most human diseases, there seems to be better understanding of cancer that might help resolve the "LNT" model. The public's concern, after being exposed to radiation, is the potential of producing cancer. The most rigorous hypothesis of human carcinogenesis is the "multistage, multimechanism" chemical carcinogenesis model. The radiation carcinogenesis LNT model, rarely, if ever, built it into their support. It will be argued that this multistage, multimechanism model of carcinogenesis, involving the "initiation" of a single cell by a mutagen event, followed by chronic exposure to threshold levels of epigenetic agents or conditions that stimulate the clonal expansion of the "initiated" cell, can convert these benign cells to become invasive and metastatic. This "promotion" process can be interrupted, thereby preventing these initiated cells from transitioning to the "progression" process of invasion and metastasis.

Keywords

multistage carcinogenesis, errors of DNA repair, errors of DNA replication, organ-specific adult stem cells, epigenetic mechanisms, tumor promotion, cell–cell communication

> "Unfortunately, the inherent limitations of epidemiology make it extremely difficult to directly quantify health risks from these exposures. . . . Interactions between radiation epidemiologists and radiation biologists will become increasingly important as the field focuses more on the effects of low doses of radiation."[1]

Introduction: What Can Radiation Carcinogenesis Learn From Chemical Carcinogenesis That Might Resolve the Issue of the Linear No-Threshold Hypothesis

Acknowledging the context of the current state of concepts, derived from the Human Genome Project, Precision Medicine, experimental results and concepts related to the study of carcinogenesis and use of modern sophisticated technologies, artificial intelligence and multiple epidemiological studies, practical public policies, related to known or suspected factors related to human cancers, still must depend on incomplete information. Given that there has been no rigorous resolution of the question: "Is there a threshold or not for radiation, as it pertains to the induction of cancer?" Since the early observations that individuals exposed to radiation were at risk of cancer, the widespread therapeutic use or the large numbers of individuals exposed to the atomic bombs in Hiroshima and Nagasaki only heightened the concern that any exposure might increase the risk of cancer. Even in the case where radiations were used to treat cancers, there always was the concern that the "side effects" of that therapy, while also leading to non-cancer effects, would also increase the risk for the induction of

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new cancers. The practical limitations for experimental testing of very low level acute or chronic exposures, as well as different dose rates, prevented the resolution of the problem. For a number of other reasons, even in the thorough studies of the many humans exposed to the atomic bombs,[2] there is no unequivocal answer that there is or is not a threshold for radiation-induced carcinogenesis.

Since the assumed "driving factors" in radiation carcinogenesis has been the induction of gene and chromosomal mutations, almost all of the experimental animal studies focused on the molecular and cellular in vitro and in vivo mutation assays. However, while additional problems have clouded even those results, namely, these various in vitro and in vivo mutation assays were themselves not easily interpreted. Each one of these assays, such as the thymidine kinase minus assay, sister chromatid assay, hypoxanthine-guanine phosphoribosyl transferase assay, and Pig-a (phosphatidylinositol N-acetylglucosaminyltransferase, subunit A), had built-in artifacts that prevented clean interpretation that "positive" results always meant a true mutation had resulted in the phenotype recovered.[3-5]

However, even those problems appear not to be the main deterrent in resolving the issue of thresholds or not. Underlying the concept that radiation "causes" cancer is that the radiation-induced genomic DNA damage that led to the mutation was all that was needed to produce that cancer and the "cancer stem cell."[6] Rarely, if ever, in those early radiation carcinogenesis studies, does one find discussions of other nonmutagenic mechanisms.

It is here that one sees that the 3 different fields of studying the induction of cancer, namely, radiations, chemicals, and microbes (viruses, parasites, and bacteria), have not paid much attention to each other. This author, having been trained in radiation-induced DNA damage/repair and mutagenesis at Oak Ridge National Laboratory, then switched fields to study chemical carcinogenesis at the McArdle Laboratory for Cancer Research at the University of Wisconsin and went to the Radiation Effects Research Foundation-Hiroshima/Nagasaki to examine the atomic bomb survivor results, saw that the experimental mechanistic studies and concepts, which came out of the chemical carcinogenesis studies, were not being used to design or interpret radiation carcinogenesis studies.

Only a few radiation carcinogenesis studies, using the major concept that came out of the multistage, multimechanism concept of chemical carcinogenesis, had been reported.[7,8] Specifically, this chemical carcinogenesis concept led to 3 distinct phases that occurred in a single normal cell when it was converted to an invasive, metastatic cancer cell.[9-11] These 3 phases were referred to as the "initiation" phase, the "promotion" phase, and the "progression" phase. Clearly, from the chemical carcinogenesis understanding of the 3 phases, the "initiation" phase was caused by an "irreversible event" or possibly a "mutagenic" event, whereas the "promotion" mechanism was a nonmutagenic or "*epigenetic*" mechanism. Yet looking at the finding of the atomic bomb survivors in 1990,[2] it was apparent that the acute exposure to ionizing radiation was significantly affected by other nonmutagenic or "epigenetic" factors.[12-15] So, it now becomes apparent that it is not a question that "Does radiation contribute to the multistage, multimechanism carcinogenesis process?", but "Which step or steps can acute radiation, at any dose/rate level, contribute to the carcinogenic process?" At this early stage of this discussion, since ionizing radiation can induce chromosomal mutations,[16] can an irreversible event, might it be the "initiator" in any cancer found in the exposed human? Might ionizing radiation be a "promoter"? If so, are each of these steps with or without "thresholds"?

Can the Resolution to the "LNT" Versus the "Threshold" Hypotheses Be Done With More Experiments?

The history of the biological effects of the exposures of radiation and chemicals has been hotly debated by proponents of the linear versus the threshold concepts.[17,18] These opposing concepts have been supported by numerous experimental and conceptual arguments.[19-22] Somehow a linear no-threshold (LNT) concept seems to have influenced regulatory agencies around the world, in spite of (1) serious errors in the "scientific" evidence used to support the LNT concept, (2) questionable personal interpretation and support of these interpretations, (3) the lack of the incorporation of modern experimental and conceptual understanding of human pathologies associated with exposures to these physical and biological effects, and (4) even alleged unprofessional behavior of some of the major players in this important debate that has influenced global public policies on the use/exposures to these agents.[17]

Rather than adding to or criticizing all the efforts to examine the personal and social events that have taken place in the historical examination of these 2 opposing hypotheses as to how exposures to a "one hit" or protracted but identical total exposures, low-dose exposure, varying dose rate or concentration-dependent exposure, or any other variable that has been studied, an attempt will be made to answer the question: "What experiment(s) need to be done today to help resolve this important public health issue?" The simple, but brash and concise, response to this question is: *No experiments need to be done, because the answer already exists in the scientific literature*. While not all human toxicological mechanisms of molecular or biological end points (mutations, cytotoxicities, and epigenetic alterations of gene expressions) or their roles in all pathologies are known to date, the example of human cancer will be examined from the standpoint of supporting or not the LNT hypothesis.

No One "Thing" Causes Human Cancers

When one examines the public's concern about exposures to any known or suspected toxic agent (radiation, chemicals, and viruses), 2 of their major worries are: "Will it cause birth defects in my children"; "Will I get cancer?" Since the amount of scientific research on cancers seems to overwhelm the amount of research on any other human pathology, the

experimental findings on genetic, molecular, cellular, and animal factors in the carcinogenetic mechanisms seem to strongly support the contention that these toxic factors do not support the LNT concept. In addition, while not as much research has been done on birth defects, there seems to be some sharing of common mechanisms leading to a toxic agent's ability to be involved in the carcinogenic process, as well as in the teratogenic process.[23]

Some of the early observations of the formation of cancers has led to the operational concepts that carcinogenesis is a "multistep, multimechanism process," consisting of the "initiation" of a single cell in the body, followed by the amplification or "promotion" of that single "initiated" cell into a benign clone and the subsequent conversion of one of these cells into an invasive and metastatic malignant cancer cell, the "progression" step.[9-11,24] This evolutionary process requires very different underlying molecular, biochemical, cellular mechanisms, which includes mutagenic, cytotoxic, and "epigenetic" mechanisms, affecting a number of genes in the nuclear and mitochondrial genomes.[25]

From the operational level of these 3 steps of carcinogenesis, it appears that all cancers are the result of being "initiated" from a single cell in the body (stem cell hypothesis).[26-34] By the time a tumor is identified, it is already a collection of cells with different genotypes and phenotypes.[35] There are 2 very fundamental questions that need to be identified at this point: (1) "What is the mechanism of this operational 'initiated' cell?"; (2) "Are all the cells (the estimated numbers being about a couple hundred trillions[36,37]) in the human body 'target' cells to be initiated?".

Operationally, the initiation stage seems to be "irreversible." This suggests that the molecular mechanism might be "mutagenesis." However, as can be noted, a stable "epigenetic" mechanism might cause a gene to be stably expressed or repressed. (To define the term, "epigenetic," as being able to alter the expression of a gene at the transcriptional, translational, or posttranslational level is important at this point)

It is here that one of the contentious criticisms of what constitutes a mutation. While, until recently, mutations were classified by altered phenotypes of cells growth and treated in vitro, such as the hypoxanthine-guanine phosphoribosyl transferase assay or 6-thioguine- resistant phenotype; the Na/K ATPase or ouabain-resistant phenotype; the thymidine kinase-resistant phenotype; or the PIG-a (phosphatidylinositol N-acetylglucosaminyltransferase, subunit A), assay. In other words, by growing normal cells in vitro and exposing them to any presumptive "cancer-associated" chemical or physical agent, one could recover from the wild-type cells, cells that have the resistant phenotypes. In the past, an agent has been interpreted as "mutagenic" or "genotoxic," if it induced the resistant phenotypes. Other "genotoxic" end points, also, have been used, such as the "sister chromatid exchange" assay[38] and the "micronuclease" assay.[39] The classic use of bacterial in vitro assays to detect mutations, which originated in the paper, "Are carcinogens mutagens,"[40] will not be discussed here because bacterial cells are simply not cellular surrogates for human cells.

Unfortunately, 3 of these phenotypes *in vitro* mutation assays (TK-, 6-thioguine resistant, and PIG-a assay) can be the result of either a real mutation or by an epigenetic repression of the gene.[3-5] Most of the interpretation of these results, when these end points are used, never make the alternative explanation, other than that these phenotypes are the result of only mutations.

Another "red herring" in the classification of an agent associated with the formation of cancer is that some agents are "very weak" mutagens. When normal population of cells is exposed to such an agent, such as phenobarbital, phorbol ester, and Dichlorodiphenyltrichloroethane (DDT), a few resistant phenotypes can be found. Rarely, in the interpretation of these kinds of results, there is a possibility that this class of "weak mutagens" is thought of as nonmutagenic chemicals that promote the preexisting, spontaneously initiate cell. Probably, the example of lung cancers found in persons never having been exposed to tobacco smoke, either as a smoker or a downstream target.[41-43]

The issue here is the assumption that the pyrolytic chemicals from tobacco smoking are "carcinogenic" or "mutagenic." While there are hundreds of chemicals found in burning tobacco, and many are known to induce oxidative stress, induce macromolecular damage, including DNA damage, and when tested in these imperfect in vitro genotoxicity tests, many are interpreted as "positive." In early tests of many of these "carcinogenic" compounds, such as benzo(a)pyrene, as well as other animal "carcinogens," that is, 7,12-Dmethylbenz[a]anthracene, results challenged the assumption that they were "mutagenic."[44,45]

Further, the epidemiological studies that indicated the risks to lung cancers in smokers were dramatically reduced after stopping smoking.[46] In other words, if years of smoking had caused irreversible mutations in the lung cells, one would not be able to explain why the stopping of exposure to these chemicals would stop the lung carcinogenic process. A more reasonable interpretation is that a spontaneous mutation in a cell, which "initiated" that cell, was "promoted" by long-term, threshold-enhancing clonal expansion of that cell until exposure to these nonmutagenic chemicals was terminated (more on this idea later). Clearly, the "promotion" process itself can be interrupted or possibly even reversed.[47]

In a study that is rarely cited, Thilly showed that, after analysis of the molecular changes found an oncogene in lung cancers of smokers and nonsmokers, the molecular change seen in the mutated oncogene was identical.[41] One reasonable interpretation is that the identical mutation in the oncogene found in both the smoker and nonsmoker seems to be a spontaneous mutation, caused by an "error of DNA replication," not due to an "error of DNA repair" of DNA damage caused by the pyrolyzed tobacco. These 2 means of inducing mutations are rarely mentioned when the roles of mutations are invoked in the pathogenesis of any disease, including cancer. The demonstration that the human skin cancer-prone syndrome, xeroderma pigmentosum,[48] which lacks the ability to repair UV light–induced pyrimidine dimers in the DNA of exposed skin cells,[49] has genomic mutations found in these cells[50,51] and that the

mutations are associated with the lack of repair of these pyrimidine dimers.[52] The mutations in the cells of this cancer-prone syndrome are referred to as "errors of DNA repair." It must be mentioned that UV light exposure to the skin leads to the most frequent form of cancer, skin cancer. All of us, especially light-skinned individuals and individuals living and working in strong UV light areas of the globe, have initiated skin cells, but not all of sun exposed have skin cancers. This suggests that just having "initiated cells" in any organ does not necessarily lead to cancer. Initiation of a single cell, with the mutation that is the first step in the multistage, multimechanism process of human carcinogenesis, must be followed by the promotion step.

On the other hand, the Blooms syndrome,[53] another cancer-prone syndrome for a wide variety of different types of cancer, has been shown to form mutations as a result of "errors of DNA replication."[54] In other words, every time a cell in this syndrome replicates, there is a finite chance a mutation is the result of an "error of DNA replication." This would be especially the case if that cell was an adult organ-specific stem cell (more on this later).

One important observation that has to be considered is the results of the atomic bomb survivors of Hiroshima and Nagasaki. It is generally assumed from the early studies that led to the interpretation that exposures to ionizing radiation could cause "mutations." Conceptually, a mutation can be classified as a point or gene mutation, and there are also chromosomal mutations of a wide variety, such as chromosome deletions, chromosomal additions, rearrangements, aneuploidy, translocations, and so on. In addition, both in vitro, in vivo animal experimental cancer studies and the epidemiological studies of radiation-exposed human cancers are "associated" with the exposure to these radiations.

In the atomic bomb survivor study, while leukemia was associated a decade after in young children who were exposed, breast cancer showed up in women who were exposed at a young age later in life.[2] One of the major reasons for this observation is that the background frequency for breast cancers in Japan at that time in history was extremely low. Since the background frequency was so low, any slight increase that was seen was shown to be attributable to the radiation exposure. If the atomic bombs had been exploded in a region where the background frequency to breast cancer was extremely high, the same frequency of breast cancer induction by ionizing radiation would not have been statistically attributable or even seen. Therefore, it is highly doubtful that in the atomic bomb survivor study, the increases in breast cancers were not simply due to the result of ionizing radiation induction of "initiation" step in the carcinogenic process but rather due to some other mechanisms.[14,15,55-58]

Several experiments have shown that when ionizing radiation is used as an initiator in animals, then followed by promotion process, it led to the interpretation that ionizing radiation is a rather "poor" initiator.[7,8] Even in the animals, where a few cancers were found, one could reasonably argue that the ionizing radiation exposures actually "promoted" preexisting "initiated" cells.

Lastly, one must fit the role of viral agents that have been associated with various cancers, such as SV40, human papillomavirus, hepatitis virus, and so on.[59-61] Also, the genetic engineering of various oncogenes, such as Ha-Ras, SRC, Neu, and so on, does not lead to transforming a single human cell to a metastatic cell.[62] Again, these viruses or oncogenes do not mechanistically contribute to all 3 steps of carcinogenesis. The fact that in vitro human cell experiments have clearly shown that no one has malignantly transformed normal human cells with a virus or oncogene.[63,64] To provide an explanation for these results, discussion of what is the cell of origin for the initiation process to start (see discussion later on the "Stem Cell" versus "Dedifferentiation" hypotheses).

If Ionizing Radiation Is a Rather Poor "Initiator," How Does One Interpret the Appearance of Cancers After Exposure? The Roles of Spontaneous Mutagenesis in Adult Organ-Specific Stem Cells and of Tumor Promotion

Since all human beings have "initiated" cells in all our organs, regardless of how the mutations in these "initiated" cells were caused, and only 1 out of 3 of us get cancer before we die, how do we explain this reality? The simple answer is that those who were diagnosed with cancer were "promoted" by some endogenous or "exogenous" agent or condition. That demands our understanding of the characteristics of the promotion process. *First*, from the classic animal promotion process,[65,66] the promoting agent had to be given after an initiation event occurred.[67] *Second*, the promoting agent could be an endogenous chemical, such as a hormone,[68] growth factor,[69] cytokine,[70,71] or an exogenous agent, such as a pollutant, pesticide, dietary agent, or pharmaceutic agent,[72,73] to which the amount given had to exceed a "threshold"[74-77], and *third*, to be exposed for a regular, sustained chronic period of time.[78]

Fourth, promotion also seems to be both gender and species dependent.[79,80] *Fifth*, these promoting agents, while able to induce oxidative stress,[81] were not genomic DNA damaging or mutagenic agents.[24] *Sixth*, promotion can also be accomplished by stimulation of cell/tissue growth and inhibition of apoptosis[82,83] by cell death, cell loss, or cell compensatory hyperplasia.[84]

This latter point needs to be explained. In human somatic, progenitor, and differentiated cells, there are 2 sources of DNA in the cell, namely, genomic and mitochondrial DNA. As will be discussed later, the stem cells (embryonic, germinal, and adult organ-specific stem cells) have little mitochondria.[85-87] Therefore, if one detects DNA lesions in the cell of an exposed tissue, such as the liver, one must determine if that damaged DNA was in an adult organ-specific stem cell, progenitor, or terminally differentiated cell. In addition, one must determine if that DNA was genomic DNA or mitochondrial DNA of that type of cell. One early observation and interpretation was that a powerful skin tumor promoter induced oxidative stress[81] and

was a mutagen because it inhibited "error-free" DNA repair.[88] It should be apparent by now that these tumor-promoting endogenous or exogenous agents are not DNA damaging or mutagenic agents. Phenobarbital, polybrominated or polychlorinated biphenols, 2,3,7,8-tetrachlorodibenzodioxin, DDT, and so on, are not mutagenic, not initiating, or not genomic DNA-damaging agents.

Seventh, promoters all have threshold levels needed to stimulate the growth of the initiated cell, as well as to inhibit the apoptosis of the initiated cell.[74-77] The promotion process selectively amplifies the initiated cells to form benign lesions, such as papilloma in the skin, enzyme-altered foci in the liver, polyps in the colon, or nodules in the breast. These are clones of the original single "initiated" cell.

Eighth, the promotion process must also be done in the absence of "antipromoters," which can act to blunt the action of any oxidative stress of the promoting agent. Promotion is, in many cases, associated with inflammation,[89-91] which suggests that the promoting agents, by triggering immune cells to secrete various cytokines, which, in turn, act as tumor promoters of any initiated cell in solid tissues.[92]

Now, in the case of nonsmokers having lung cancers never having smoked or been in the presence of downstream smoke, these cancers could have originated via initiation of a single cells by an "error of DNA replication" but were promoted by chronic exposure to other endogenous or exogenous agents.

What Is the Origin of the "Initiated" Cell That Is Promoted?

This question is extremely relevant to the issue of whether there exists an LNT or threshold relationship to the induction of human diseases, such as cancer after exposure to radiation, chemicals, or viruses. In all 3 cancer-associated agents, a one-time (one hit) or chronic exposure must bring about the 3 operation steps of carcinogenesis, namely, "initiation" of a single cell, the promotion of that single cell into a benign tumor, and the ultimate conversion of one of those promoted and initiated cell into an invasive and metastatic cell. These "operation" steps are mechanistically very different. If, for example, a single photon of an ionizing radiation damaged the specific genes that are responsible for all 3 steps, that is, it causes that single cell to proliferate and not die by apoptosis and to become "immortal," invasive, and metastatic (the so-called hallmarks of cancer[93,94]), then the LNT concept will have been scientifically satisfied.

Therefore, since one of the first phenotypic changes that must occur during the first, one hit event in the cell that becomes, initiated, primed for promotion and invasive/metastatic properties, it has been assumed to be the induction of "immortalization." The early work on oncogenes suggested when a specific oncogene is genetically engineered into a population of normal, primary cells in vitro, a few cells are termed "immortalized," but not neoplastically transformed.[62] These "immortalized" cells can be, then, transformed by other oncogenes (ie, Ha-ras) or other agents to become neoplastically transformed. In other words, these 2 distinct steps by 2 different molecularly acting oncogenes mimic what is seen *in vivo* in the classic "initiation," "promotion," and "progression" process of carcinogenesis.

The 2 major and diametrically opposed hypotheses of carcinogenesis, the stem cell[28-34] versus the "dedifferentiation" or "reprogramming"[95] hypotheses, have had a long period of supporters and challengers. Only with the recent demonstration of the isolation and characterization of human stem cells (embryonic,[96,97] induced pluri-potent,[98] somatic nuclear transfer,[99] and organ-specific stem cells,[34,100-104]) has there been some clarifications between the 2 opposing hypotheses. Embryonic stem cells, by definition, can form teratomas when injected back into an adult animal. This suggests that when embryonic stem cells are placed in an improper microenvironment during development, they do not differentiate normally into organized tissues, but they can differentiate into bone, teeth, hair, and so on. Yet, if these teratoma cells are placed back into a normal embryonic blastocyst, they can differentiate in a regular fashion.[105]

One of the recent major scientific demonstrations, showing a few pluripotent stem cells can be found after a normal population of primary human fibroblast cells are exposed to a specific set of genes (Oct3/4, Sox2, Klf4, c-Myc), led to a Nobel Prize to Dr S. Yamanaka.[106] His interpretation of this discovery was that these 4 transcription factor genes were able to "reprogram" differentiated somatic cells to become "embryonic-like" and could form teratomas when put back into an adult animal. Yet, the interesting characteristic of these induced Pluripotent Stem (iPS) cells is that they carry the differentiation marker genes of the tissue from which they were isolated.[107-110]

There are several challenges to the interpretation of these iPS cells. If, in vivo, in the human being, a somatic differentiation cell can be "reprogrammed" to be a noncancer embryonic, pluripotent stem cell by a "one hit" exposure to a photon, single chemical, or a biological agent, that interaction must mimic the molecular events that the 4 transcription factors bring about when they potentially create iPS cells. That, of course, is the "one" hit. But is it? The explanation is that these "iPS" stem cells are the result of a minimum of 4 independent gene expressions to bring about this "reprogramming" to the embryonic-like stem cell state. These reprogrammed somatic differentiated cells should form teratomas in the adult human body. Since most human adult cancers are carcinomas or sarcomas, how could "reprogramming" explain this fact? Obviously, since in vitro studies to induce neoplastic human cells from normal primary human cells have not been found after multi-exposures to many "carcinogens,"[63,64,111] this could suggest that the "one-time" exposure was insufficient to attain all the "hallmarks of cancer",[93,94] or of the 3 operation steps of the multistage, multimechanism process of carcinogens, namely, mutagenic "initiation," epigenetic "promotion," and stable irreversible "progression."

The alternative explanation of the production of "induced pluripotent" stem cells is that, when the 4 Yamanaka

transcription factors are introduced to a population of very early primary human cells, which contain a few, rare organ-specific adult stem cells, only the few adult stem cells, which already express the Oct4A gene but do not express the connexin genes or have functional gap junctional intercellular communication, will be able to survive the normal replicative limitation of progenitor or non-stem cells.[25,112-117] That is, with the integration of an *exogenous* set of embryonic transcription factors, especially Oct4A, together with their already expressed *endogenous* Oct4A, only these few rare cells survive crises. The frequency of these recovered iPS cells seems to be similar to the number of adult stem cells in that early primary population of cells. It has been noted that the recovery of induced pluripotent stem cells is dramatically reduced with late passaged primary cultures. The reason for this is probably that these cultures are normally grown in 20% oxygen in medium with limited antioxidants. Since the Oct4A gene is a redox-sensitive gene,[118-120] multiple passages in high oxygen would induce differentiation/apoptosis of these few adult stem cells, thereby explaining why the Hayflick phenomenon is associated with the limited passage levels seen with primary cultures of human cells.[121] Human stem cells and human diploid fibroblast cells, grown under low oxygen tension and in high antioxidant levels, seem to have an extended passage level.[122,123]

If the first step of the multistage, multimechanism, that is, "initiation," is the result of an irreversible event in a single adult stem cell, that irreversible step is functionally blocking its ability to terminally differentiate or become "mortal." One must recognize that any stem cell is naturally "immortal" or have an extended life span to divide symmetrically or to "self-renew." Once it is induced to divide asymmetrically, one daughter is maintained to self-renew, while the other daughter differentiates or becomes "mortal." Therefore, the "initiation" event must be one that blocks terminal differentiation or "asymmetric division". It blocks "mortalization".

This now leads to another major challenge to the role of viruses in the human carcinogenic process. Obviously, as the Nobel Prize recipient, Dr H. zur Hausen, noted, many viruses have been associated with various human cancers.[61] Yet the oncological-associated viruses cannot bring about the full "hallmarks of cancers" or fulfill all the 3 operational steps of the initiation, promotion, and progression steps of carcinogenesis. One of the terms used in viral carcinogenic in vitro experiments is that there are "immortalizing viruses." That comes out of those studies where normal in vitro human primary populations of cells are infected with these viruses or transfected with viral "oncogenes."[59-61] In a manner similar to the Yamanaka, when embryonic transcription genes are put on primary populations of human cells, only a few "immortalized" cells are found.

The alternative explanation or challenge to the concept of "immortalizing viruses" is that while the viruses or oncogenic viral oncogenes might infect all cells of the primary culture, only those rare adult stem cells that had the virus would have their ability to divide asymmetrically blocked. In those cases, only these normal "immortal" adult stem cells would be blocked from "mortalizing" or terminally differentiating. Experimentally, this has been seen by Land et al[62] when the myc gene was put into primary cells in vitro. These cells were, themselves, not neoplastically transformed but were now "immortalized" or in this new interpretation, the few normal but "immortal" adult stem cells were unable to "mortalize" or terminally differentiation. If correct, the term, "immortalizing" viruses should be changed to "mortalizing-blocked" viruses. These viruses, then, could be viewed as "initiators" in the multistep, multimechanism carcinogenic process. As in the Weinberg experiments, these viral "initiated" cells now have extended life spans, where other events/steps can meet the required "hallmarks of cancer."

Implications to the Use of LNT Interpretation of the Hiroshima/Nagasaki Atomic Bomb Survival Attributable Cancer Data to Global Radiation Standards

If one considers that human cancers are the result of (1) the "initiation"/"promotion"/"progression" process; (b) originating from a single adult organ-specific stem cell; (c) a gene mutation, caused by either an "error of DNA repair" or by an "error of DNA replication" that blocks the "mortalization" of the stem cell during the "initiation" step; (d) ionizing radiation being a rather poor point mutagen; (e) the sustained clonal amplification of the single initiated cell by epigenetic promoters at threshold levels, in the absence of "antipromoters" to form a benign lesion; and (f) multiple gene and chromosomal mutagenic and epigenetic changes in a single "initiated" cell that brings about the required "hallmarks of cancer," allowing invasion and metastasizing of that cell to become a "cancer stem cell," then it would seem evident that an LNT concept cancer does not explain cancers attributable to the acute exposure to the atomic bombs. In other words, the cancers seen in the atomic bomb exposed survivors could have been the result of the acute radiation affecting one of these 3 operational stages of carcinogenesis.

Stepping back from the actual data on the atomic bomb survivors, one knows that the control population, who only were exposed to "background" radiation, as well as thousands of chemicals (smokers, drugs, dietary factors, hormones, etc), psychological, social, and cultural influences (exercise, working at night, stress), and biological agents (bacteria, parasites, viruses), could be victims of a wide spectrum of cancers.

Clearly, while the pattern of cancer and other chronic diseases seems to vary from culture to culture, as well as within each culture/ethnic group, one finds a small fraction of these populations who are clearly genetically predisposed, the epidemiological evidence points to powerful roles of various environmental or behavior influences that govern to appearance of these chronic diseases. Nutrition and diets, either caloric restriction[124,125] or caloric excesses,[126] appear to be a major modulator of the appearance of these chronic diseases. Probably, there is no better example of how the incidence of a given

cancer, such as breast cancer, can be influenced by changing marriage patterns,[127] nutrition and diet[128,129] and alteration of diet modulating microbiome populations,[130-137] is that of the effect of both migration of Japanese and the change in their diet with their traditional living environment of the dramatic change in breast cancer incidences.

In the Japanese culture, at the time of the 2 atomic bombs and before, the patterns on the state of health and the phenotype of the Japanese population was quite unique, in that they were small in stature, had a rather uniform type of diet (absence of red meat, abundance of rice, vegetables, raw fish, tofu, green tea), and men who smoked but women who did not smoke. The first 2 types of cancer that appeared after exposure to the atomic bombs are leukemia in children and breast cancers in women who were exposed at a young age.[2]

With that as a generalized background, the major observation was statistically attributable breast cancers noted in the survivors because the background frequency in Japan at that period of time was extremely low. Therefore, any small increase above that low background frequency was noted. Today, the frequency of breast cancers in Japan is approaching that was seen in Western countries because, among other factors, the young are eating the Western diet. Had the atomic bomb survivors been eating the Western diet prior to and after the exposure to the atomic bombs, that small attributable increase due to the exposure to ionizing radiation would not have been noted.

Therefore, the fundamental question that has to be answered is: "Why was the breast cancer frequency in the Japanese population so low prior to and immediately after the bombs were dropped?" and "Why is the frequency changing in present-day Japan?" The simple answer seems to be a dramatic change in their diet and caloric intake. One needs only to see that the median life span of those old Japanese was among the highest in the world, especially the women. The role of low caloric intake, by itself, is a significant risk reducer of many chronic diseases.[138] It is known that the general Japanese population of preatomic bomb events was significantly calorically restricted.[139]

Of course, the reduction of calories might be accompanied by either a reduction of vital vitamins and minerals, as was the case with the European World War II prisoner population or alternatively it could be done with a diet of antioxidant-containing foods and sufficient protein, during the World War II in Japan, where tea, soy products (tofu, natto), vegetables, and raw fish were available. Therefore, the caloric restriction seen in Europe might have predisposed a more disease-prone state and increased morbidity and mortality, whereas in Japan, one might predict life span expansion and reduction in risk of chronic diseases.

Significant amounts of soy products were consumed by Japanese women for generations before the bombs were dropped. Consequently, both the Barker hypothesis[138] and caloric restriction might have contributed to the frequency of attributed radiation-induced breast cancers in the women survivors of the atomic bombs.[2] From a basic biological standpoint, normal human adult breast stem cells have been shown to be induced to differentiate when exposed to genistein, a component of soy products.[140] With a reduced number of breast stem cells in female offspring of mothers who eat significant amounts of soy products, these women would have less breast tissue after puberty and fewer breast stem cells as targets for the initiation process. Furthermore, these women would also be calorically restricted after any initiation or exposure to the atomic bomb radiation, which would probably negatively affect the promotion of any initiated breast stem cell. Equally important, it has to be considered that the role of diet could modulate (increase or decrease) the number of breast stem cells during development that could modulate the risk to cancer later in life by increasing or decreasing the "target" size of potential "initiated" cells, caused by either an "error of DNA repair" or an "error of DNA replication."[141,142]

Since human adult breast stem cells have been isolated and partially characterized,[143] it has been shown that they could be differentiated by many agents, including genistein, a component of tofu. If during pregnancy of the female fetus prior to the dropping of the atomic bombs, dietary genistein and other dietary chemicals sharing the same properties might have forced the adult breast stem cells (and possibly the bone stem cells) in the developing female fetus to prematurely terminally differentiate or apoptose. These female babies would have been small at birth, and when they reached puberty, they had few breast stem cells, on which her hormones would need to make breast tissue. At the time of the bomb, these young women would have few adult breast stem cells to be "targets" to start the carcinogenic process. Since ionizing radiation does not seem to be efficacious "initiators,"[7,8] the attributable breast cancers in these young Japanese women might have been the result of some gene mutations caused by "errors of DNA replication". Various hormones, growth factors, and cytokines[68-71] have been shown to stimulate stem cell proliferation, thereby forcing a finite production of mutations via "errors of replication". Those that received high, but nonlethal doses, might have had perturbations of their immune system to trigger inflammatory factors.[144] The inflammatory factors are known to be "promoters" of the carcinogenic process.[90,91] Counteracting the promotion of any initiated stem cell for the female, the absence of smoking, a known tumor promoter,[145,146] must be considered. Even for the many Japanese men who are smokers, their risks to lung cancer seem to be dramatically reduced, compared to American men who smoke less, by the high level of drinking green tea.[147] Green tea components have been shown to reduce the effects of various chemical tumor promoters.[148,149]

Conclusions

This example of how the appearance of any cancer, which must conform to the "initiation," "promotion," and "progression" process, can be modulated (increased or decreased) at each step by increasing or decreasing the number of adult organ-specific stem cells, by increasing or decreasing the mutation or

"initiation" step, by stimulating or inhibiting the proliferation of the promotion step, and by enhancing or prohibiting the progression or invasion/metastatic steps. In other words, to be blunt, a "one hit" or LNT concept of cancer production would be impossible. No one agent can "cause" cancer by fulfilling all the underlying mechanisms, which are distinctly molecularly different (mutagenesis is not like epigenesis). This would be particularly impossible at very low exposures to radiation, acutely or chronically. The formation of DNA damage can be repaired or not. DNA repair in the organ-specific adult stem cells also seems to be better at repairing DNA damage than their differentiated offspring,[150,151] although further studies must be done to establish specific results under different condition of various types of stem cells. This seems clear after the results of differential cell death of small versus large intestinal stem cell results after ionizing radiation in situ.[152] Promotion of the initiated cell needs "threshold" amounts of agents, for long sustained periods of time in the absence of antipromoters. It seems that no further experiments need to be performed to see if very low doses of radiation or concentrations of toxic chemicals can induce cancers in an LNT fashion. In the absence of rigorous evidence that radiation carcinogenesis is qualitatively different, mechanistically, than chemical, viral carcinogens, or radiation carcinogenesis, radiation-induced human carcinogenesis must conform to the multistage, multimechanism process of carcinogenesis. Assuming that to be correct, the LNT model must be rejected. In addition, one of the major implications of this hypothesis is that radiation carcinogens must conform to chemical carcinogenesis, in terms of having 3 distinct phases of "initiation," "promotion," and "progress." As important, the use of the atomic bomb data as the "gold standard" for determining the scientific foundation for public policy of radiation exposure standards must be seriously challenged, as it is now very clear that caloric restriction, nutrition, and diets play a major role in modulating the carcinogenic process by radiation, chemicals, or biological agents.

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