Towards a unifying theory of late stochastic effects of ionizing radiation

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\textbf{A B S T R A C T}

The traditionally accepted biological basis for the late stochastic effects of ionizing radiation (cancer and hereditary disease), i.e. target theory, has so far been unable to accommodate the more recent findings of non-cancer disease and the so-called non-targeted effects, genomic instability and bystander effect, thus creating uncertainty in radiation risk estimation. We propose that ionizing radiation can give rise to these effects through two distinct and independent routes, one essentially genetic, termed here type A, and the other essentially epigenetic, termed type B. Type B processes entail envisaging phenotype as represented by a dynamic attractor and radiation acting as an agent that stresses cellular processes leading to the adoption of a variant attractor/phenotype. Evidence from the literature indicates that type B processes can lead to the inheritance of variant cell attractors and mediate a category of trans-generational effects quite distinct from classical Mendelian inherited disease, which is type A. The causal relationships for radiation-induced somatic human health detriment, i.e., cancer and non-cancer (e.g., cardiovascular) disease, are discussed from the point of view of the proposed classification.

This approach unifies at a fundamental level the heritable and late somatic effects of radiation into a single causal framework that has the potential to be extended to the effects of the other environmental agents damaging to health.

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1. Introduction

In the 1930s Müller and Timofeeff-Ressovsky demonstrated that X-rays were capable of causing heritable changes in the offspring of fruit flies. Their colleagues, Zimmer and Delbruck employed target theory to estimate the physical size of the cellular component responsible for transmitting the change induced by the radiation [1]. Target theory has since provided the underpinning for classical radiobiology, first being applied in the work of Crowther [2]. It connects the loss of the biological activity with the number of interactions of ionizing radiation in a particular volume (target) of the biological material. An important assumption of target theory is that one primary random ionization leads to a change in structure of a molecular component of the target, thus causing an altered functionality [3].

Since 1953, with the discovery of the DNA structure and subsequently the genetic code, radiobiological dogma has assumed that the late stochastic effects of radiation, initially thought to be only cancers and hereditary disease, were the result of mutations in the genomic DNA: the specific sequences of the code that were relevant to the endpoint being studied were the target for radiation effects. For single-gene hereditary conditions obeying Mendelian rules, this dogma is confirmed by the specific-locus tests in mice [4,5], but for cancer the hypothesis remains unproven. Subsequently, non-cancer diseases, e.g., circulatory disease, have been added to the late effects caused by radiation exposure as a result of epidemiological information from the survivors of the atomic bombings in Japan [6] and from radiotherapy patients [7]. In addition, the inheritance of mini-satellite mutations induced in humans [8–10], potentially mirrored by the inheritance of mutations in TDRL induced in mice [11,12], have been observed. These mutations are produced at rates in excess of those normally found for DNA sequence mutations. However, their connection to health detriment has yet to be established.

In 1992 the phenomenon of genomic instability in the clonal descendants of haemopoetic cells, showing a high level of non-clonal chromosomal aberrations was uncovered [13]. At the same time the bystander effect in the Chinese hamster ovary cells was described, manifesting itself as an unexpectedly high percentage (30%) of cells with sister chromatid exchange, when only 1% of the cell nuclei were estimated to have been the target for alpha-particle passages [14]. To compare: genomic instability describes the delayed radiation effects in the irradiated cell and its progeny, whereas the bystander effect describes the response...
of non-irradiated cells to the targeting of a neighbouring cell by radiation. Both effects are thought to be important, in terms of dose effect, at low doses. For genomic instability the magnitude of effect is such that based on target theory it cannot be caused by mutational damage to specific gene sequences related to the biological endpoint [15]. Due to the absence of a causal connection with a specific cellular target these effects have been termed ‘non-targeted’. Therefore, an intensive search is currently underway to find a new paradigm that does not rely solely on a relationship between specific gene mutations and biological effects, to underpin the consequences of exposure to ionizing radiation [16].

The possible basis for such a unified theory of the late stochastic effects of ionizing radiation, covering hereditary disease, cancer and non-cancer disease is explored herein. It is argued that while radiation can act to cause health damage through the mediation of specific mutational damage there is a second process in which the organizational, rather than the material, properties of the living cell respond to the deposition of energy from ionizing radiation.

2. Materials and methods (model)

2.1. Envisaging the cell as an open system and the classification of radiation effects

The phenomenon of order or organization per se has received relatively little attention in conventional scientific thought, the concept of entropy and statistical mechanics and then in a probabilistic context, being almost the only examples. However, in biology some diseases can be seen as attributable more to failures in organization than to damage to the substance of the cell/organism. One can take the diseases involving protein mis-folding as an example. It is known that the protein conformational landscape can be very broad and defined by the protein environment. The conformational substrates adopted by a protein are a result of the evolutionary selection of states that are needed for protein function [17]. Failure of peptides to adopt the proper conformation or to be processed appropriately is associated with many human pathologies [18], for example Creutzfeldt-Jakob disease, Alzheimer’s disease, Parkinson’s disease, and cystic fibrosis [18]. It has been proposed that mental retardation associated with irradiation is due to disruption of organizational processes of the brain in antenatal development [20]. This should not be surprising as altered neuronal organization is also found in several major psychiatric disorders [21]. Therefore, changes in biological organization at different levels, from molecular to the organism, may be crucial to the processes that underlie radiation-induced disease more generally. In fact we will show that the late effects of ionizing radiation, in addition to being caused by damage to the material of the cell, can also be caused by organizational events. We will relate these two categories to Aristotle’s material and efficient causes, respectively [22].

Ionizing radiation gives rise to sub-cellular energy-deposition events, initially in volumes of a few nanometers in diameter, which are generally fully resolved, physically and chemically, in the time scale of seconds to minutes after energy deposition, within the same cell. In the longer time frame and in multi-cellular organisms, the consequent biological processes will involve other cells and other tissues in the organism. In this paper the cell is chosen to serve as the primary organizational unit and we will consider the processes that take place within a cell to change it and the consequences of these changes for the system (tissue/organism) in which that cell resides. Another important feature of the cell as an organizational unit is its openness when it itself is viewed as a system. Each individual cell is open not only thermodynamically, in respect of its nutrients, for example, but also in a wider sense: it is driven by influences from its environment. Here we treat the cell as such a thermodynamically open system [23,24].

Traditionally the cell is presented in biology as a semi-autonomous carrier of mutable and heritable elements, genes, which define its phenotypic properties. Despite correctly depicting genomic DNA as the receptacle of information defining the material components of the cell, this model does not allow much scope for explaining how organization of information is stored and processed, except in terms of transcriptional regulation via chromatin marking. This view also does not explain both the plasticity and simultaneous robustness of the cellular phenotype, which is manifested in the processes of ontogeny [25] and cell differentiation [26]. Moreover, it ignores the openness of the cell and fails to give a satisfactory explanation of the non-targeted effects of radiation. We assert that these problems can be overcome by viewing the cell as an operational dynamic component of a tissue/organism (system) in which it resides. An important feature of such a vision is that the cellular phenotype is derived from the interacting network of active gene products, modified by environmental signals, including those from other cells, from the physical environment and the changes in levels of DNA transcription products prescribed by the genotype. Such interacting networks are termed attractors (Fig. 1).

This dynamic and highly interconnected intracellular network is self-organized [15,27–30]. The stable states of the dynamic systems are called attractors because states in their immediate vicinity drain into them, thus endowing the stable state with a degree of robustness or resilience to perturbation. Cellular phenotype (in this context a quasi-stable state of a cell) is represented by such attractors. Kaufman has shown that randomly constructed Boolean networks exhibit self-organized state cycle attractors under a specific range of conditions of connectivity [31,32]. Self-organization is commonplace in far-from-equilibrium (thermodynamically open) systems [33] and self-organization was established by Alan Turing as the explanation for morphogenesis [34]. Recent biological discoveries confirm the importance of self-organization and pattern formation for basic cellular events, like DNA replication, chromosome segregation, microtubule assembly and even organization of the major cellular organelles [35]. The phenomenon of self-organization can be regarded as a prerequisite for the emergence of biological functions.

An attractor can be seen as the manifestation of multiple dynamic steady states between dynamic modes of a dynamical system, in this case the active gene products encoded in the genomic DNA of the cell. Evidence consistent with phenotype being represented by attractors has been found in bacteria [36] and in mammalian cells [37]. The attractors may possess a high degree of robustness against perturbation

Fig. 1. Presentation of the cell in a tissue, as a system built on interaction between genome signals and environmental signals in terms of the primary normal influences upon it. Environmental signals are presented as composed of the signaling from the interacting tissue attractors and the signals outside of the surrounding tissue (marked with dashed line). All these signals influence and regulate the cell attractor, which is a dynamic, highly interconnected, and open system of all the cellular components and biochemicals (see Supplementary material for details).
Despite being based on an active protein network with a continuum of gene-product activity, they can be usefully approximated by simple Boolean network models, where gene-coding sequences take only the value of either “on” or “off” and in positional interaction networks “active” or “inactive” [38,40].

Both the material and the organizational aspects of the perturbation of cellular function can be combined into a unified view of the cell, by assigning processes triggered by specific changes in genomic DNA as type A processes, and those triggered by stress, for example, caused by the need for the cell to repair damage in general to its genotype, which leads to perturbations of the cellular organization, as type B processes [41,42]. This categorization effectively resolves the material causes of change (type A) as genetic effects of radiation and the efficient causes of change (type B) as epigenetic effects, using the term epigenetic in its generic meaning of “over and above” genetics rather than it being associated with specific manifestations such as chromatin marking. An overview of the categorization of the effects of radiation in this way is summarized in Table 1.

It is useful to define exactly what we mean by cellular phenotype. Phenotype is defined here in general terms as the linear sum of the morphological and functional, including regulatory and signaling, features of the cell. The stable-over-time loss or gain of one phenotypic feature would represent a phenotypic change.

For completeness we should mention a third category of radiation-induced effects, namely deterministic effects, in which loss of function of a tissue or an organ is due to interphase or proliferative cell death. These effects occur predominately at high doses and relatively shortly after exposure. Cell death may be programmed as in apoptosis in which case it may be assumed that cellular attractors leading to apoptosis are the cause. Additionally, equilibrium (the lowest available energy state) is an attractor for any living system and thus in the context of a cell this would mean the absence of any functional activity, that is, any organized state for protein activity. This type B process would lead to dead cells in the tissue and potentially to necrosis.

In this putative unified theory the cellular phenotype of an established species is assumed to have been evolutionarily conditioned and its attractor is referred to as the home attractor [15]. The conditioning has optimized the attractor for genomic stability (integrity of replication of the genotype) and robustness (resilience to perturbation). The transition to a genomically unstable phenotype is assumed to be mediated through the adoption of a variant and previously unoccupied and thus unconditioned, attractor which has the potential to develop into an inherited disease (if germ cells) and into somatic disease (if somatic cells) [27]. The genomic instability phenotype can be regarded as an incomplete phenotype in that its relative lack of robustness means that it is relatively easily perturbed, thus readily adopting further variant phenotypes, as is seen from the experiments of Falt et al. [43]. As the genomically unstable attractor is by definition displaced from the optimum state regarding genomic stability, the unstable phenotype will be a mutator phenotype generating further genotypic variation. Given the number of active gene products involved in a typical human cell attractor, once displacement to a variant attractor has occurred the process will be irreversible and can only be stopped by elimination from the tissue or constraining the growth of abnormal phenotypes, e.g. abnormal cells. For further elaboration of these processes see Supplementary material.

We now consider type A and type B processes separately both in germ cells and somatic cells.

3. Results

3.1. Type A processes

The traditional basis for molecular biology is embodied in its central dogma, namely that information is transferred from the coding sequences in the genomic DNA, the genotype, to the functional protein structure [44]. and, therefore, DNA sequence defines the biological function, i.e., phenotype. Modification of the parental germ-cell genotype can result in modifications to the inherited phenotype in the offspring. The generation of phenotype from genotype was believed by many to be a deterministic process, which was the intellectual basis for the human genome-sequencing program, the first results of which were published in 2001 [45]. However, there was an obstacle in establishing direct deterministic causality in that the numbers of the known proteins and their variants were found to exceed by three to five times the number of protein-coding sequences [46]. This indeterminism of the cellular proteomes is mostly due to various transcriptional and post-transcriptional events, such as alternative splicing patterns specific to a cell type [47], condition-dependent protein folding [17] or more than 30 types of post-translational modification [48]. This complexity illustrates the dependence of phenotype on epigenetic regulation through the deployment of...
coding sequences rather than simply on the properties of the coded product.

3.1.1. Germ cells

A radiation-induced single gene-sequence mutation is inherited according to Mendelian rules, namely, that in relation to the descendants of the irradiated parent the disease is “diluted” with each succeeding generation so that the chances of an individual inheriting the effect are progressively reduced. It is generally assumed that such damage can lead to hereditary disease and it is indeed the basis for risk limitation deployed by the International Commission on Radiological Protection (ICRP), based on data from specific locus mutations in mice [49,50].

3.1.2. Somatic cells

It is widely believed that radiation-induced cancer is initiated and driven by DNA mutations, and is, therefore, a type A process. Indeed, the somatic mutation theory (SMT) of cancer attempts to explain carcinogenesis in somatic cells by the same mechanism as is applicable to the hereditary effects discussed above. Historically, SMT defined the steps of carcinogenesis as the accumulation of genetic changes, initiated by the formation of a specific triggering mutation fixed in the progeny and leading to the acquisition of further mutations and the so-called “hallmarks of cancer”, for example, anchor-free growth and senescence [51,52]. In the early clonal stages of this process the problem is viewed exclusively as a cellular and not a tissue problem. Nevertheless, cancer is characterized not only by an accumulation of aberrant cells, but also by altered tissue organization and signaling [53].

Relatively little is, however, understood about the multi-step process that intervenes between the infliction of the initial damage to the cell and the appearance of cancer. Cancer cells are associated with complex chromosomal damage [54] and have a mutator phenotype [55], that is, they acquire spontaneous mutations at a higher frequency than do normal cells. The complexity of this process tends to obscure the identity of the initial mutations in the mature cancer, the stage at which it can usually be diagnosed and examined. However, genome-wide sequencing has shown that the link between a specific mutation and a cancer is tenuous in that the process tends to obscure the identity of the initial mutations in the mature cancer, the stage at which it can usually be diagnosed and examined. However, genome-wide sequencing has shown that the link between a specific mutation and a cancer is tenuous in that link between a specific mutation and a cancer is tenuous in that specific types of cancer rarely carry the same complement of mutations [56–59]. This has generally been interpreted as indicating several potential “pathways”, one of which may apply in any specific case. Today, in the field of cancer biology, the SMT is being replaced by models that take into account, among other factors, epigenetic processes and the influence of the surrounding tissues [60–62].

3.2. Type B processes

Type B processes emerge in our model from the perturbation of the cellular attractor beyond its limits of robustness, leading to a transition in phenotype independent of any phenotypically relevant change to the genotype; thus a purely epigenetic effect. The conditions under which this can happen have been defined previously [27].

As noted above, genomic instability is what might be called an incomplete phenotype, in the sense that due to the weaker robustness of the variant phenotype further transitions to other variant attractors are likely, leading to migration between variant attractors, as shown by the radiation-induced increase in the diversity of transcription patterns in genomically unstable cells [43]. Thus, the process of genomic instability can be seen as the cell transiting a succession of variant attractors which modify the functions of the cell itself and of its progeny. Among the functions that can be lost by an affected cell is the correct signaling mode to adjacent cells. In this way recipient cells in the same tissue can be destabilized and themselves adopt the instability phenotype [63].

3.2.1. Germ cells

Genomic instability, in the form of mini-satellite mutations in humans [8–10,64] and TRDL mutations in mice [11,12], has been observed in the offspring of male parents exposed to radiation at the spermatogonial stage. There is, however, some ambiguity as to the precise nature of these circumstances. Male germ cells are more susceptible than those of the female, but some studies suggest that spermatogonial cells are most sensitive whereas others suggest later stages of spermatogenesis are more sensitive. See Ref. [65] for a discussion on this point.

The mutations can take the form of differences in the number of repeats in the particular DNA sequences. These sequences are only a small fraction of the total genomic DNA and as such would represent a small target and thus require very high radiation doses to “hit”. In fact, the yield of these mutations in relation to dose is far higher than for sequence mutations, the target for which is the coding sequence of a gene.

Repeat sequence mutations are apparently not inherited in a Mendelian manner, but rather transmitted down the male germ-line; when an F1 male mouse with a parental irradiation history is mated with a normal female the offspring will be affected, but if a female F1 mouse with a parental irradiation history is mated with a normal male the F2 will be normal [12,66]. This can be interpreted as indicating synchronization of the pro-nuclei in the zygote, as would be expected when two dynamical systems share the same environment [67].

In some cases instability (e.g., TRDL mutations) is detectable in the germ cells of the parent, but in other cases what is transmitted is not material damage but instability only in terms of the modified or variant attractor, and only later this becomes manifest in terms of material damage. For example, in another case of inherited instability, i.e. the reversion of the pink eyed mutation (pun) in mice, a white coat with black spots in the F1 progeny of a male parent irradiated at the spermatogonial stage indicates reversion of pun during embryogenesis, whereas a black mouse would indicate reversion in the parent [68]. However, if TRDL mutations are present in the offspring, other damage, for example, increased DNA breaks and increased mutational frequency at the Hprt locus, are also observed in somatic cells [11].

It is not clear whether or not inherited genomic instability leads to hereditary disease, but one study suggests that it may. In this study, dominant lethal mutations inherited from an irradiated male parent skipped a generation, and the F2 progeny of F1 males mated with females without a paternal radiation history showed an excess yield of dominant lethal mutations [69].

3.2.2. Somatic cells

A primary feature of somatic cells is the huge range of highly specialized functions they perform, including, as a prerequisite to live cooperatively in communities as tissues and organs, the ability to signal to neighbouring cells. As noted above, the transition to genomic instability is more likely to mean loss of critical functions rather than acquisition of new functions. Such losses pose a threat to the functionality of the tissue. It seems highly likely, for example, that the bystander effect [14] is simply a response to aberrant signaling from an unstable neighbouring cell rather than a specifically “directed” effect. Thus, for example, in some studies, where the bystander response is apoptosis, BE is seen as a protective effect [70], and in others where it is associated with increased mutation rate [71,72] it can be seen as potentially detrimental to health.

It also seems likely, given the frequency with which instability can be induced by ionizing radiation in vitro, that there are processes by which aberrantly behaving cells in tissue can be
eliminated or in some way constrained, by the action of neighbouring cells. Thus, as is observed, the frequency with which both genomic instability and bystander effect are observed in vivo is expected to be much reduced compared to the rate in vitro [73,74]. Possible evidence for the above derives, for example, from post mortem examination of the tissues of individuals who died of trauma. Increased frequency of non-symptomatic pre-cancerous lesions [75] was revealed without the victims having any other sign of cancer. Were such lesions to develop into malignancies, cancer incidence rates would be much higher than those observed in their age group. It has been proposed that these foci are surrounded by slowly dividing so-called cancer stem cells and are essentially constrained from further development unless disturbed [76].

In the context of cells in tissue, type B effects can be divided into two categories depending upon whether or not the aberrant cells have a selective growth advantage. Where there is such an advantage it is proposed that, if the constraints to further growth fail, clones of cells with a growth advantage and an enhanced propensity to migrate to other variant attractors can develop. It should be noted that due to the mutator phenotype of genomically unstable cells, DNA sequence mutations will also be acquired. These modifications will occur stochastically and having occurred may endow some of the hallmarks of cancer [51]. Mutations will also prevent the reversal of attractor transitions, thus ensuring that the process is progressive.

An implication of this type B mode of carcinogenesis will be the absence of molecular markers that might predict the outcome; the process is a stochastic one generating consequential mutations. The predictions of such a model are very close to the reported situation reflected by the results of genome-wide sequencing of cancers: close examination of the mutations in several cases of histologically identical cancers shows that no single mutation is a driver of the process [56–59].

Several lines of evidence support epigenetic initiation and development of cancers. For example, the implanting of mouse medulloblastoma cells into a blastocyst can result in normal development and loss of the malignant phenotype, indicating that in this case sequence mutations are not involved [77]. In carcinogenesis, changes in the epigenetic phenomenon of chromatin marking may precede and promote genetic damage [78,79]. Therefore, mutations associated with cancer may be largely consequential on the mutator phenotype of an unstable cell. Indeed, the mutations per se may not even be required for long-lasting cancerous metabolic changes in inflammation [80]. The above suggests that epigenetically based organizational events (type B processes) are potentially important players in radiation-induced carcinogenesis and possibly in carcinogenesis in general.

A second consequence under the type B category would arise if the initially genomically unstable cell did not have a growth advantage over its neighbouring cells but was modified phenotypically, so as to change some of its functions and to cause aberrant signaling. This could lead to transitions from the home attractor to variant attractors in neighbouring cells. If this process were progressive over time a focus of cells with variant attractors/phenotypes (not necessarily of a single type) could arise and if these variant phenotypes lacked critical functions they could result in malfunctioning at the tissue level (Fig. 2).

What should be emphasized in the case of the type B processes, both in relation to cancer and non-cancer disease, is that although the initial transition from the home to the variant attractor may be relatively common, the final outcome, tissue sufficiently extensively modified in a functionally damaging way as to constitute health threat, may be both comparatively rare and significantly delayed in relation to the initiating radiation event. Indeed, from in vitro studies it seems likely that the initial step in this process is relatively frequent (see above) but that in the majority of cases the aberrant cells will be eliminated or their aberrant signaling will be blocked in the tissue by the concerted action of normal cells. Moreover, the transition to a variant attractor does not necessarily imply either aberrant signaling or loss of relevant function, i.e., disease-causing conditions; it may be entirely benign in character although it is envisaged as being in a process of constant “evolution”. Thus, while the initiating events could be comparatively frequent the outcomes in terms of disease could be relatively rare although the probability of their becoming relevant is constantly increasing with time, as in effect these processes are a component of the aging process.

The development of atherosclerosis illustrates the involvement of organizational components in potentially radiation-triggered non-cancer pathology. Atherosclerosis is one of the non-cancer pathologies that are thought to be induced by exposure to ionizing radiation at low doses [81,82]. Its origin lies in a malfunction of the endothelial cells lining the blood vessels. Endothelial cells serve to control access of agents carried in blood to the underlying tissue layers, the intima and smooth muscle, and they normally form a contiguous layer on the inner surface of the blood vessel. The earliest indication of atherosclerosis is an inflammatory response due to build-up of low-density lipoprotein under the endothelial layer due to increased permeability of the lining [83]. Through a relatively well-understood sequence of events this lesion develops into a plaque, which may break up and lead to myocardial infarction or stroke. There are two main theories for the development of atherosclerosis, namely the “inflammatory response to injury” hypothesis and the “monoclonal/mutational” theory. The former postulates that a lesion in the endothelial cell layer, caused perhaps by shear stress or some agent like nicotine that allows the leakage, is the origin of the condition. The latter postulates that a mutation in the endothelial/smooth muscle cells develops into a lesion that has many properties in common with cancer. Support for the latter hypothesis derives from the monoclonal nature of the plaque [84,85]. However, the monoclonality alone may not be indicative of a cancer-like process as smooth muscle cells appear to develop in clonal patches [86].
We suggest here a possible mechanism involving as the initial step the transition of a cell to a variant, genomically unstable, phenotype without a selective growth advantage. Andreassi et al. [87,88] have reported the evidence for the involvement of genomic instability in atherosclerosis. We suggest that genomically unstable cells with a modified phenotype involving the loss of critical morphological and signaling functions, thus adversely affecting neighbouring cells, could have a reduced ability to exclude unwanted material from the intima and thus could be the originating event for atherosclerosis.

As is already assumed for cancer but is not yet clear for non-cancer diseases [89], the relationship of the disease condition to the radiation dose would be contingent on the effect in a single cell (followed by signaling processes independent of the radiation exposure to enroll other cells into an appropriately dysfunctional state). In this case the dose response would be expected to be linear at low doses. In addition, a long latency might be expected as the initially affected cell may have to undergo several phenotypic transitions and adjacent cells become similarly affected through aberrant signaling, before the functional loss became a relevant disease condition.

4. Discussion

Since the uncovering of the phenomenon of radiation-induced genomic instability and the bystander effect in 1992 there has been a greater level of uncertainty in estimating the health risks of exposure to ionizing radiation. The primary cause of the uncertainty was the lack of a theoretical framework underpinning the effects of low doses of ionizing radiation where genetic mechanisms (type A processes) were shown by the target theory not to be relevant and where direct evidence from epidemiology is difficult to obtain for rather fundamental reasons. The unified approach outlined above provides a model that can be further tested, but that is already consistent with evidence from experimental biology. The unification is achieved at a fundamental level, i.e., in terms of causality relating to cellular phenotype. It is not usual to invoke Aristotle's final cause in biology because of the possible implication of teleology, but we assert here that the maintenance of stably replicating species is the final cause and that it implies a very high level of integrity is maintained through replication of the genotype. Maintaining the integrity of the DNA, a relatively easily degradable molecule under physiological conditions, requires the cell to undertake extensive "housekeeping" activities, the correct performance of which is vital to the maintenance of the attractor that determines phenotype and thus functionality at the cellular and tissue levels. The capacity to perform these housekeeping activities is conditioned by the evolutionary experience of the cell and if exceeded can cause an attractor transition from what we term the home attractor [15] to a variant attractor that is by definition less able to maintain the genotypic sequence; this is the genomically unstable phenotype. Such transitions are type B processes. As the attractor is the efficient cause of phenotype, the type B processes are independent of the type A processes, which turn on the identity of the functional proteins, the material cause. In essence therefore the unification is achieved by including in the overall cause of phenotype the usually neglected efficient cause.

The strongest evidence for a distinction between type A and type B processes is seen in the context of germ cells and the two distinct modes of germ–line inheritance in mice, namely Mendelian for type A processes and patrilineal for type B processes. That a type A process applies in humans is undecided. If radiation induces heritable sequence mutations the survivors of the atomic bombings in Japan would be expected to show it. The initial analysis showed no evidence of hereditary disease [90], however a more recent analysis contradicted this result and a further re-analysis is in progress (personal communication from Dr. Roy Shore). However, no evidence of genomic instability has been found in the survivors of Japanese bombing [91,92]. A new study reports no evidence of increased mutation at micro-satellite loci in the offspring of the Japanese bomb survivors [93]. On the other hand, there are examples of other exposed populations where inherited genomic instability has been found and others where it has not [65].

In somatic cells the question of whether cancer is a type A or type B process is unresolved. Much evidence exists to suggest that it is primarily type B, with the undisputed association with mutations being a consequence of the mutator phenotype rather than a cancer-triggering, i.e. causal event. However, some cancers, for example thyroid cancer, exhibit little evidence of an early genomic instability, perhaps because thyroid cells divide only slowly. The evidence that circulatory disease is associated with low dose exposure to radiation can be described as "indicative" [7,81], however, it is notable that genomic instability has been detected in atherosclerotic plaque [88] and could be evidence of a type B process.

In acting as a cause of both cancer and non-cancer disease, radiation is not unique. Tobacco smoke causes both lung cancer and circulatory disease, both presumably as a direct result of the action of the products of tobacco use on the relevant cells. In addition traffic pollution, which has been shown to cause genomic instability in mice [94] is associated with increased cardiovascular disease [95–97]. Thus, genomic instability can be seen as a common factor in health-damaging environmental agents, suggesting that the type B process may be a generalized stress response. Indeed, in the TDRL mutational experiments treatment of the male parent with ethynylstroureca produces similar results to ionizing radiation in the offspring, although the type of DNA damage induced by the two agents is quite different [98].

The question arises how the proposed unifying framework can be tested. In the classical radiobiological dogma, hereditary disease is either the result of mutational and chromosomal damage (reciprocal translocations etc.) or it is of multi-factorial origin, that is, radiation acting together with other environmental factors in ways that are poorly understood. Cancer is assumed to be initiated and progressed by sequence mutations in the genomic DNA, initially as the result of the radiation exposure and subsequently spontaneously, at high dose rates and possibly also by exposure at low dose rates. Non-cancer disease is regarded as deterministic and caused by loss of cellular function at the tissue level resulting from cell killing.

However, none of these "mechanisms", except for single locus mutations and chromosomal damage, are in any way proven as the route from irradiated cells to a disease endpoint; rather there is a degree of plausibility to the narrative explanation in terms of the radiation-exposure conditions that have been demonstrated to give rise to the effects. It is no coincidence that the hereditary effects mediated by single locus mutation and chromosomal damage are better understood than the somatic effects, since in the latter case numerous cell divisions intervene between the irradiated cell and the endpoint, where, as noted by Russell, the former are a "trace" effect [99].

Thus, much support for the unifying model can be garnered from the available evidence, for example, that radiation-induced non-cancer diseases such as cataract of the eye [100] and circulatory disease [7] are seen in statistically significant excess at lower doses than would be the expected incidence if cell killing were the cause. It is also notable that atherosclerotic plaque exhibits features of genomic instability as well as classical genomic sequence mutations [88].

That radiation-induced cancer could be epigenetically, rather than genetically, initiated is perhaps the most contentious aspect of the model. Radiation causes both mutations and genomic insta-
bility and these are also both features of cancer. Thus, a *priori*, the genetic and epigenetic mechanisms can be considered as on an equal footing. There is, however, an important difference in that if a specific mutation or a specific DNA damage is the initiating event it should be present in all cancers so initiated, that is, there would be a “signature” at the molecular level: the same would not be true of a cancer initiated by genomic instability. The early thyroid cancers in those exposed as children to Chernobyl fallout are identifiable as “radiation-induced” because sporadic cancer is so rare in that age group. However, careful study has shown that there is no “radiation signature” in such cancers [101]. A detailed evaluation of these two candidate mechanisms will be given elsewhere.

Teratogenesis (birth defects), which can include mental retardation, and multi-factorial inherited disease [102] have not been considered so far. On the basis of the very scarce information on the mechanisms of this process, we have assigned it potentially to both type A and type B processes in developing tissues of the embryo, as well as to the possible genetic damage to the germ cells, according to Wilson’s principles (see Table 1).

Finally, assessing the risks entailed in type B processes will require a different methodology. One can measure molecular features in unhealthy cells, but deducing the nature of the processes that led to that condition is much more difficult and may require not only new methodologies but also a modification of the approach to investigation at the scientific level.

5. Conclusion

The phenomenon of radiation-induced genomic instability and the growing epidemiological evidence for delayed health effects even at low doses of radiation strongly favor mechanistic considerations other than the traditionally considered cell killing or induction of DNA mutations. Under the unified model, cellular processes can be envisaged as robust and at the same time flexible with the interactions of both the environment and the genotype. This allows us to merge the mechanisms and cell-organizational damage into one model and to propose the link between these early events and the late radiation-associated somatic health effects, such as cancer and cardiovascular disease, as well as the hereditary effects in the germ-line.

Conflict of interest statement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mrgentox.2010.11.003.

References


