Radiat Environ Biophys (2000) 39:79–87

REVIEW ARTICLE

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Molecular mechanisms of radiation carcinogenesis and the linear, non-threshold dose response model of radiation risk estimation

Received: 18 October 1999 / Accepted: 10 February 2000

Abstract Recent research in molecular radiation carcinogenesis is reviewed with the specific aim of exploring the implications this research may have on the dose response relationship of radiation-induced cancer at low doses and low dose rates. It is concluded that the linear non-threshold dose response hypothesis may be used in radiation protection planning as a simple, convenient method to optimize procedures and regulations, but should not be mistaken as a stringent scientific conclusion directly derived from the present state of knowledge of the processes involved in radiation carcinogenesis.

Introduction

Ionizing radiations are an established cause of cancer. Exposure of different populations to high doses, most often accumulated over a relatively short period as a result of war and accidents, from occupational exposure or as part of diagnosing or treating disease has increased their cancer incidence and their cancer mortality. Epidemiological data on these populations remain the most important basis for our understanding of radiation risk in man. It should be explicitly stated that any hypothesis on mechanisms, on dose and on time dependence or on type of radiation-associated disease which is not consistent with the available epidemiological data has to be considered unfounded. Epidemiological data remain the benchmark of risk assessment. It is only epidemiological data that provide information on the types of cancer which radiation may induce: in particular acute and myeloid leukaemias, all types of lung cancer, breast cancer, papillary thyroid cancer, stomach cancer, colon cancer, but *not* rectum cancer, *not* cervix cancer, *not* chronic lymphocytic leukaemia [1]. It is only epidemiological data that provide information on the dependence of tissue sensitivity to the carcinogenic action of radiation on the age at radiation exposure (such as the extraordinary change of the radiosensitivity of the thyroid gland by an order of magnitude during the different stages of childhood [2]), or the dependence of the incidence of radiation-induced cancer on attained age. Finally, it is only epidemiological data which provide information on the size of the risk at high doses, in excess of 0.5 Gy accumulated over days, months or a few years.

This article is based on a keynote lecture presented at the First International Conference of the World Council of Nuclear Workers on 'The effects of low and very low doses of ionizing radiation on human health' in Versailles, June 1999

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The limits of epidemiology

Despite the outstanding role that epidemiological data play in radiation risk assessment, there are problems which epidemiology cannot resolve, neither at present nor in the forseeable future: Epidemiological data *cannot* quantify the cancer risk from long term low intensity radiation exposure which is characteristic for regulated occupational exposure of radiation workers.

As long as no fingerprint alterations have been identified which would clearly distinguish a radiation-induced cancer from the same type of cancer induced by other environmental, dietary or metabolic carcinogens – and there is little hope for that at present – any attempt at quantifying the cancer risks associated with occupational radiation exposure within the generally accepted limits remains elusive. In an editorial which accompanied the publication of the second analysis of the British National Registry for Radiation Workers [3], Doll [4] stated: "Some might argue that...the conclusion will always be that larger numbers and more research are needed. In a sense that is true. Sooner or later we have to base our estimate of risk at very low doses on some model whether

derived from laboratory experiment, epidemiological evidence of the dose response relationship at higher doses or, better, a combination of the two". He continued that the ongoing studies on radiation workers in Europe might establish a trend down to very low doses of less than 200 mSv – however, that would only indicate that there is some risk at those doses but *not how much risk*. The extrapolation to *how much risk* requires mathematical models which can be fitted to the epidemiological data. The results of this extrapolation depend little on the actual data points at those low doses but more on the mathematical structure of the model. Although most epidemiological and experimental studies use only two or three different models, the number and complexity of mathematical models that can be used to fit the epidemiological data is theoretically unlimited – and so is the resulting range of risks at low radiation doses. It is rare that certain mathematical models can actually be excluded from further consideration because their fit is statistically improbable such as the linear non-threshold equation which does not fit the leukaemia data from the Japanese A-bomb survivors [5] or the discrepancy between the surprisingly low observed leukaemia rates among the liquidators in the Russian Registry and the expected risk in leukaemia rates which had been calculated on the basis of the A-bomb survivor data [6]. In an accompanying editorial, Boice [7] concluded that the risk of leukaemia predicted from models based on studies of high dose and high dose rate exposures are not consistent with the observed numbers of leukaemia seen among liquidators. Considering various reasons for this discrepancy such as overestimation of radiation doses in liquidators or underreporting of cases, he finally stated that "risk from low doses delivered at low rates may be much less than predicted from higher doses at higher dose rates, possibly because of the opportunity for cellular damage to be repaired". As epidemiology reaches its limits it turns to radiobiology for help!

Besides the extrapolation to low dose or low dose rate exposures, the translation of the results of epidemiological findings in one population to another population may pose serious problems to epidemiology. Rates of different types of cancer vary enormously between populations, even between closely related ones [8]. In radiation epidemiology it is common to group all types of non-leukaemia cancer together and analyse the dose dependence of these global cancer rates. This does not make sense to an oncologist. The different types of cancer are just too different with regard to their causes, to other risk factors, to their natural behaviour, to their age at manifestation, their curability, the influence of genetic factors, etc., and so probably is their dependence on radiation exposure. Radiation is only one of numerous, potentially interacting carcinogens to which we are all exposed: the most important carcinogens are products of the physiological metabolism in our body or derived from the diet we eat and drink [9]. Others can be identified as being due to lifestyle or ethnic customs such as cigarette smoking, excessive sunlight exposure or betel nut chewing. Occupational exposure to carcinogens particularly in some traditional professions such as farming and building may affect people more seriously than environmental exposures. In order to incorporate all these potentially carcinogenic factors into one comprehensive model of carcinogenesis which permits the numerical estimation of risk at low intensity exposures (of radiation or chemical carcinogens or their combination), the knowledge of the molecular and cellular processes has to be translated into mathematical models. Such models have recently received wide attention in radiation protection. Many scientists hope or expect that these biologically founded mechanistic models might improve the precision of risk estimation at low doses and dose rates and the risk transfer to populations with different baseline cancer rates.

Another serious problem which so far eludes epidemiological methods is the question of how to allow for the unquestioned existence of smaller or larger subgroups of people with increased genetic susceptibility to cancer. Only radiobiological considerations and models of population genetics can help to resolve the associated questions [10].

The multistage process of carcinogenesis and models of radiation carcinogenesis

In the last 10 years, the understanding of the cellular and molecular processes that eventually lead to the different types of cancer has increased in an explosive mode. Some simple principles have emerged and have already entered into some models of radiation carcinogenesis, although the large number of complex and modifying factors have not yet been assessed quantitatively and the predictive value of the models is very limited. This refers in particular to extrapolations beyond the actual data, which, however, is the real purpose of building such models, particularly the estimations of risk after low doses accumulated over long periods of time. It should be recognized that the mechanistic models of radiation carcinogenesis essentially use probabilities for the transfer of a cell or tissue from one stage of the carcinogenic process to the next. Yet, according to Wilson [11], who considered the carcinogenic risks from chemicals, "the only argument to support the hypothesis that thresholds do not exist relies on an assumption that all the processes that act as barriers to attack by mutagens act as probabilistic barriers, and that the probability distribution is infinite. It is, however, more likely that they are truncated". This assumption of probabilities inevitably has the consequence that a threshold with zero risk is excluded a priori. In other words, the non-threshold conclusion is not the result of a scientific analysis but the a priori assumption inherent in all models used in carcinogenesis and particularly in radiation carcinogenesis.

It is generally accepted that the process of carcinogenesis occurs in a series of steps while a cell with un-

limited proliferative potential, i.e. a stem cell, acquires several heritable changes, i.e. somatic mutations to go from bad to worse. None of the individual steps causes cancer by itself. Cancer, rather, is the result of the accumulation of all the necessary changes in one stem cell which survives despite all damage accumulation. The number of these steps may vary between types of cancer (two to six) as may the sequence. These numbers, reflecting alterations in putative oncogenes or tumour suppressor genes leading to the transformation of a normal human cell into a malignant one, were almost exclusively derived from the deconvolution of the incidence-versus-age curves for different malignancies [12]. For some tumour types, such as retinoblastoma or colorectal cancer, the molecular-pathological investigations showed that the number of genetic alterations found in the tumour cells were in good agreement with the numbers derived from theory.

The best studied cancer with regard to the progression of the malignant process from a normal stem cell to invasive cancer is cancer of the colon [13, 14]. A sequence of mutations in defined genes has been identified which drive the neoplastic development through a series of morphologically distinct stages into invasive colon cancer. In some of them, the first step is a germline mutation in one allelic copy of the APC gene, making all cells of the colon epithelium more susceptible to the growth of a benign adenoma as the second copy of the APC gene and a second tumour suppressor termed MCC (for "mutated in colon cancer") is lost, usually by loss of the entire genes. This leads to the growth of a benign adenoma as the first stage to tumour growth. Genes such as APC which control and regulate the proliferation pattern in normal epithelia to meet the demands of tissue function are called tumour suppressor genes. If both copies of such a gene are damaged – most often, one is damaged or silenced (e.g. by hypermethylation) and the other lost ("loss of heterozygosity") – proliferation gets out of control although it is still far from a malignant state. However, during this progressive adenomatous growth, the cells become increasingly unstable and can accumulate further mutations: genes which have normal regulatory functions such as c-Ki-ras may become permanently activated and become oncogenes or tumour suppressor genes such as DCC ("deleted in colon cancer") or p53 may be lost until, eventually, a clone has developed through mutation and natural selection which escapes the control mechanisms of the organism and grows in an autonomous way, infiltrating neighbouring tissues and organs and seeding distant metastases. The five separate steps which were identified in the neoplastic development of colorectal tumours, each representing a mutation event [15], are in good agreement with the number of four to seven genetic alterations predicted from the theory [12]. Only recently, experimental carcinogenesis has demonstrated for the first time that alterations in three defined molecular mechanisms (telomerase activity, regulation of cell division and bypassing of the apoptosis machinery) are not only essential, but sufficient to change human fibroblasts and epithelial cells from a normal phenotype towards a fully malignant one [16]. For radiation biology, the crucial question is, which of the different steps can be induced by radiation.

The empirical/mechanistic models of multistage carcinogenesis which are in use to investigate effects of radiation are strongly influenced by these observations on multistage carcinogenesis in colon cancer. The main driving force for initiation and progression is the mutations which lift a stem cell from one stage to the next. However, in the more recent models [17] complex interactions have been incorporated with clonal expansion, programmed cell death and cellular differentiation, all of which have decisive effects on the progression of malignant development. In the mathematical models, these processes are represented only by vectors and probabilities. Yet, in order to understand what is really going on we have to examine each of these biological processes in some detail and how it may affect the malignant progression of an initiated stem cell.

Oncogenes and tumour suppressor genes

Many of the genes which if mutated cause progression of the carcinogenic process by a further step have recently been characterized with regard to their normal function and the change of function that occurs as a result of the mutation. Research in this field is very competitive and new results are being reported almost daily. The associated gene products belong to a variety of metabolic pathways of normal cell biology although most of them take part in the regulation of cell cycle progression and proliferation (although gene functions normally unrelated to cell proliferation have also been associated with genes involved in the carcinogenic process, such as genes encoding for proteins which play a role in DNA repair such as BRCA1 [18] or BRCA2 [19]). Operationally, two different types of mutations are used to classify these genes: (1) those where a mutation causes a gain of function – they are called proto-oncogenes before mutation and oncogenes after mutation – and (2) those where mutations cause a loss of function – they are called tumour suppressor genes.

In the development of colon cancer, the loss of function of the APC tumour suppressor gene is the first step. Loss of function of tumour suppressor genes is assumed to be the first step in the majority of solid cancers, whereas in the development of leukaemia and lymphoma the first step appears to be the activation of a proto-oncogene into an oncogene, e.g. by translocation of a promotor besides the active site of a normally repressed growth promoting gene site [20]. About 50 gene specific chromosome translocations have been characterized at the molecular level in human leukaemias and lymphomas [10]. In contrast to the generalization mentioned above, very specific gene rearrangements play a key role in radiation-induced thyroid cancer in children.

Thyroid cancer is very rare in children. The massive rise in the incidence of this cancer among children in the Ukraine and in Belarus following the Chernobyl accident means that each such cancer is radiation induced with a probability of >90% [21]. This provided, for the first time, the unique opportunity to look for fingerprint mutations in tumours which would be specific for its radiation origin. However, so far, little evidence for fingerprint mutations has been provided. Yet, very specific activating rearrangements involving the ret proto-oncogene have been found [22, 23]. The PTC rearrangements are characteristic for papillary thyroid cancer and have been found in radiation-induced thyroid cancers more frequently than in spontaneous thyroid cancers which occurred in later adulthood. The PTC3 rearrangement is characteristic of early arising thyroid cancer in young children with very aggressive growth behaviour, whereas the PTC1 rearrangement characterizes a less aggressive thyroid cancer in somewhat older children with a longer latency [23]. The initial expectation that these ret rearrangements might be a fingerprint of radiation causation is probably incorrect. The most interesting aspect, however, is the specificity of the molecular mechanism which leads to the activating translocation. Since this is assumed to be an early if not the first step in the carcinogenic progression of these radiation-induced cancers, the investigation of the molecular mechanisms of the initiating radiation effect in the target cell should concentrate on this highly specific event.

Translocations and other rearrangements are typical effects of radiation on chromosomes and appear to be related to DNA double-strand breaks. Therefore, rearrangements which bring a proto-oncogene under the influence of another ubiquitously active gene are by no means unexpected – they should happen as a matter of chance just as other translocations and rearrangements occur after radiation exposure, throughout the genome. Yet, when the joining regions of the two merged genes were sequenced, an amazing degree of specificity became apparent [23]: this specificity is so high that it is incompatible with any degree of randomness which is regarded as the hallmark of molecular radiation effects. The mean thyroid dose of radiation-induced thyroid cancers in Belarussian children is <0.2 Gy [21]. This dose would produce <10 DNA double-strand breaks per cell. If we make the plausible assumption that the thyroid of very young children contains approximately 10,000 thyroid stem cells, this dose would induce a total of <100,000 double-strand breaks in the stem cells of each thyroid. The risk of radiation-induced thyroid cancer in Belarussian children is approximately 0.1%. This means that approximately 10^8 double-strand breaks cause one thyroid cancer. There is no question that with this number of double-strand breaks there is a certain probability of having two dsb's in the two critical genes which take part in the ret-PTC translocations if we assume that the total number of human genes is approximately 10^5 [24]. However, the number of base pairs in the genome is $>10^9$. Therefore the chance of hitting two specific base pair combinations with a double-strand break is 10^{-18} and is thus highly improbable. Quite obviously, these specific rearrangements cannot be the result of two randomly occurring double-strand breaks in the ret proto-oncogene and the participating other gene and their subsequent reunion at or near the break point, even if we have to assume a tremendous amount of natural selection of the active oncogene. Thus, despite the fact that radiation is known to cause double-strand breaks and genomic rearrangements, the mechanisms leading to proto-oncogene activation appear to be much more complex than just random DNA double-strand breaks and reunion. It is much more likely that repair processes such as homologous recombination repair are involved. If any of the participating genes such as ELE indeed showed suitable homology with parts of the ret proto-oncogene, a single double-strand break in any of the two genes would, in principle, be all that is necessary to cause the observed high degree of molecular specificity of the joining regions of the translocation.

The loss-of-function mutations of tumour suppressor genes, however, are much easier to reconcile with the nature of direct and indirect radiation effects on DNA and chromosomes. Whereas oncogene activations are very specific, tumour suppressor gene mutations may involve random deletions of large amounts of DNA, large parts of the gene, or the entire gene, or even more than one gene. If the other allele had suffered a mutation (such as a point mutation or silencing by methylation) which is compatible with survival and unlimited proliferation of the affected stem cell, such loss can be tolerated by the cell. For many solid tumours, tumour suppressor gene inactivation is considered to be the first step of the carcinogenic process and is commonly assumed to affect a tissue specific "gatekeeper" gene which, after loss of function permits subsequent clonal expansion of tissue specific stem cells [25]. This clonal expansion may be associated with spontaneous or induced genomic instability which increases the chances of accumulating further mutations needed for the malignant development. This first loss of function mutation of the "gatekeeper" gene probably is the rate limiting mutation. Since loss of heterozygosity mutations are characteristic consequences of DNA double-strand breaks, radiation carcinogenesis research has concentrated on this type of mutation and thus on the role and fate of DNA double-strand breaks.

The effectiveness of DNA repair

Even a single track from low linear energy transfer (LET) radiation has a finite probability of producing one or even more than one double-strand break in the DNA of a cell nucleus [26]. Therefore, the cellular consequences of a double-strand break such as loss of heterozygosity being the basic mechanism of tumour suppressor gene inactivation should, in principle, be possible even at the lowest doses and dose rates.

Although double-strand breaks will be induced even by very low radiation doses, they may be repaired very effectively by either one of two different repair mechanisms. Both mechanisms lead to rejoining of the broken double-stranded DNA; however, one mechanism uses available genetic information by a process called homologous recombination which may result in correct healing of the break [27]. In order to lead to high fidelity repair, this process depends on the annealing of the broken DNA end to its counterpart on the homologous chromosome (before S-phase) or alternatively to its sister chromatid. Perfect homology of annealing occurs regularly during meiosis. However, annealing for homologous recombination repair requires only homology over a limited stretch of base sequences, although little data are available on the amount and degree of required homology. It may occur also between unrelated genes which present only partial homology. The mechanism of homologous recombination repair, precise as it may look in the first place, may thus be a mechanism which converts DNA double-strand breaks into point mutations or specific translocations. The International Commission on Radiological Protection (ICRP [10]) concluded that radiation mutagenesis may principally proceed via DNA deletions through misrepair and misrecombination at DNA double-strand breaks.

More common, at least at high doses and high damage concentrations, is the mechanism of ligation or end-joining. A repair molecule complex consisting of the enzyme DNA dependent phosphokinase and two other proteins, code-named Ku70 and Ku80, attaches to the loose ends of the broken DNA and facilitates rejoining but without checking the correctness of the DNA sequence against the undamaged copy. Although this mechanism reconstitutes the basic structure of the DNA thread and thus permits the cell to proceed through mitosis, errors are frequent which take the form of basepair substitution, frameshift mutations or, most frequently, DNA deletions of varying size [28]. The more complex the DNA damage the more likely are those misrepair lesions [29]. A total of 13 human genes involved in the repair of radiation damage in DNA have been cloned and/or mapped; at least 5 genes are involved in double-strand break repair [10].

The patterns of DNA damage before repair which can be analysed using microdosimetric models differ considerably from the patterns of DNA damage after repair. It has been hypothesized that the precision of repair (which might be related to the relative frequency of repair by homologous recombination) improves as the concentration of primary damage (i.e. the frequency of simple and complex double-strand breaks) decreases. Yet to determine the dependence of repair fidelity on the amount of initially induced breaks would require very sophisticated and labour-intensive experiments which have not yet been done. If it could be shown that the fidelity of double-strand break repair depended on damage concentration in the DNA, it would be a strong argument against a simple proportionality between dose and cancer-initiating DNA lesions.

The role of radiation-induced genomic instability

Another mechanism which is associated with differences in the pattern of DNA damage is radiation-induced genomic instability. Until recently, irradiated cells were thought to represent one of two states: either the cell was not damaged and all its progeny would be undamaged or it was damaged and all its progeny would inherit this damage. However, it has been demonstrated that most of the apparently "undamaged" cells acquire a state of genomic instability as a result of which the probability of new but spontaneous genomic damage is increased in each postirradiation generation for many cell generations. This mechanism occurs both in vitro and in vivo. There is some evidence that it plays an important role in the carcinogenic action of radiations [30, 31]. The implications of this mechanism are controversial. Some types of delayed effects such as chromosomal rearrangements [32], gene mutations [33] or gene amplifications might lead to transformation of a surviving cell after some divisions.

The mutational damage from radiation-induced genomic instability differs fundamentally from directly radiation-induced mutational damage. Most mutations induced by radiation directly involve loss of large parts of the tested gene, leading to loss of heterozygosity. Yet most mutations induced by radiation-induced genomic instability involve point mutations and small deletions [33]. Radiation-induced genomic instability does not lead to mutations which are radiation specific but increases the rate of those mutations which occur spontaneously, probably by similar mechanisms. This conclusion from analysis of the spectrum of mutations in the progeny of irradiated cells in vitro is also supported by investigations of the molecular spectrum of mutations in radiation-induced cancers in vivo. In mouse tumours, experiments have been performed to test the hypothesis that radiation-induced tumours should demonstrate loss of larger portions of key tumour suppressor genes in contrast to virus-induced or "spontaneous" tumours in which point mutations are expected. In a study comparing alpha-radiation-induced bone tumours with spontaneous osteosarcoma or those with retroviral aetiology, large deletions in the p53 gene were only found in tumours with radiogenic origin, whereas the spontaneous or virus-induced tumours carried only point mutations (M. Atkinson, personal communication). However, large p53 deletions were detected in no more than 30% of the radiogenic cases, making the involvement of undetected point mutations in the genesis of bone tumours after alpha-irradiation a possibility. This view is supported by recent findings in 20 liver tumours which were diagnosed in a cohort of people treated with Thorotrast, in which 95% of cases showed p53 point mutations [34]. The authors conclude that the relevant genetic alterations leading to liver cancer are the result of an induced genetic instability, rather than the direct effect of the radiation exposure.

Although the relevance of radiation-induced persistent genomic instability to neoplastic development remains to be established [10], we suggest that:

- Direct induction of loss of heterozygosity as a consequence of radiation-induced complex double-strand breaks may play a minor role in radiation carcinogenesis compared with the small mutations which also occur spontaneously and which just may become more frequent as a result of radiation-induced genomic instability.
- If that conclusion is correct, any mechanism which is involved in the processing of spontaneous or "physiological" DNA damage should also modulate the severity and frequency of DNA damage which occurs as a result of radiation-induced genomic instability. Since the cell is able to repair a very high level of endogenous DNA damage without frequent mutagenic consequences, a further small increment of such DNA damage from low dose rate irradiation should, equally efficiently, be repaired. Mutation rates will only increase if due to higher dose and dose rate the capacity for high fidelity DNA repair is exceeded.
- The mechanism which induces "radiation-induced genomic instability" appears to involve a non-nuclear target [35] and upregulation of oxidative stress [36], which also is the main mechanism of metabolic DNA damage. These experimental observations are not compatible with a single hit mechanism which is the basis for the microdosimetric justification of the linear-non threshold dose response hypothesis.
- We conclude that if radiation-induced genomic instability was indeed a key mechanism of radiation carcinogenesis, a non-linear or a threshold type dose response relationship would be a plausible dose response relationship for radiation-induced cancer even if radiation-induced genomic instability itself was induced according to a linear dose response curve.

The role of protective mechanisms and of natural selection

Since the progression of the malignant process so obviously involves the accumulation of a series of well defined mutations, the mutagenic action of ionizing radiations has been particularly implicated in radiation carcinogenesis. Any increase in mutation rates has been assumed to relate to a proportional increase in cancer rates. So far, we have argued that the increase in the specific mutations required for the carcinogenic process is unlikely to be proportional to the frequency of initial complex double-strand breaks and thus to low doses at low dose rates.

An even more persuasive argument against proportionality derives from the notion that increased mutation rates may *not* be a prerequisite of or a predictor for increased cancer rates since it is natural selection that plays the most powerful role in the entire carcinogenic process. Recently, Tomlinson and Bodmer [37] argued this point very strongly and concluded that "a raised mutation rate may make tumorigenesis faster but is not necessary for tumorigenesis to occur.... Selection is surely the overriding mechanism of cellular, somatic evolution leading to cancer.... This view of carcinogenesis can...lead to considerable new insights into the carcinogenic process".

Although the mechanisms by which natural selection works in the carcinogenic process have not been elucidated in full detail, some factors contributing to it have been studied quite extensively during the last few years such as:

- Programmed cell death
- Cellular differentiation
- Adaptive responses
- Immunosurveillance
- Intercellular communication

All factors have been demonstrated to be affected by high radiation doses yet little is known about their response to low dose rate irradiation. The underlying mechanisms for all five mentioned modulating processes which may take part in natural selection of the malignant clone (as far as they have been elucidated) are not compatible with a single target/single hit mechanism and thus not compatible with linearity extending to very low doses and dose rates.

Programmed cell death, called apoptosis, is a powerful mechanism that removes not only surplus cells during embryogenesis and tissue homeostasis [37] but is also involved in the elimination of potentially deleterious, harmful cells from the organism [38]. Although not equally efficient in all organs, DNA damage induced apoptosis is well regulated and involves many different steps of damage recognition and subsequent cellular suicide responses [39]. Radiation can affect this process in two ways: it produces the type of damage which is eliminated by programmed cell death (e.g. certain types of DNA damage and mutations), but radiation can also induce and may also modulate the efficiency of this clearing process. Some genes involved in the process are also classified as tumour suppressor genes (e.g. Rb1, p53) or proto-oncogenes (such as Bcl-2, c-myc or Fos). The main regulatory processes are post-translational. There is some evidence that radiations do not only affect gene expression but also post-translational modulation. The full elimination of normal cellular control requires the abrogation of more than one step in this process since the different pathways involved in it cooperate very effectively making it highly redundant. More importantly, most of the molecular mechanisms during the execution of apoptosis are independent of gene transcription and translation but rather rely on protein interaction [40]. Both facts make impairment of apoptosis by a single hit mechanism rather unlikely.

Radiation has been shown to induce differentiation in a large variety of undifferentiated stem-like cells. The

mechanisms are not well understood, yet, but there is evidence that intercellular communication, through gap junctions or by diffusible factors, e.g. cytokines, may be involved. Stem cells which have been triggered into differentiation are as effectively eliminated from the pool of potential target cells for carcinogenesis as cells which undergo programmed cell death. Yet there is also evidence that indirect mechanisms which are induced by higher radiation doses may lead to reduced differentiation. There is abundant evidence that differentiation of stem cells is a very finely regulated biological process which responds in a flexible way to all sorts of disturbances of the normal steady state of tissue homeostasis. The role of differentiation in the carcinogenic process and its modulation by potential carcinogens such as radiation is still very speculative and solid data on dose response and reliable experimental models are lacking. Since the mechanism appears to involve a large degree of intercellular communication and intra- and intercellular signalling, no simple dose dependence and, in particular, no simple time dependence of this mechanism would be expected.

Adaptive mechanisms have been invoked to play a major role in protection against the carcinogenic process. However, the experimental models used to investigate adaptive processes are very remote from those involved in the initiation and progression of a malignant clone. The classical experiment would demonstrate that irradiation of human lymphocytes in vitro with a radiation dose of approximately 100 mGy would induce temporary radioresistance resulting in a reduced frequency of unstable chromosome aberrations after a subsequent high dose irradiation [41]. This process appears to be closely related to the observation of low dose hypersensitivity described in a variety of cell lines in vitro. Low radiation doses (±100 mGy) induce high fidelity repair mechanisms [42]. Low dose hypersensitivity might be regarded as a similar protective mechanism as the apoptotic process [42]. Although a large amount of data on adaptive mechanisms and low dose hypersensitivity have been published and some evidence for the underlying molecular mechanisms has been provided, it would be premature to speculate on their role in radiation carcinogenesis at low doses and dose rates.

The results of research into the mechanisms of carcinogenesis in general and into the effects of ionizing radiations on the different processes which are potentially involved in radiation carcinogenesis in particular has demonstrated an enormous complexity. No mathematical model has yet been designed which takes account of all these interacting processes – and it may well be that such models would be too complex and contain too many parameters so that they no longer can provide reliable and clear answers to the questions we need to ask. In view of this situation, any statement on the carcinogenic risk from prolonged very low dose rate irradiation reflects personal judgement as much as scientific insight.

The role of genetic susceptibility

The most powerful scientific tool to identify cancer-associated genes is the genetic analysis of families in which members show an increased susceptibility for specific cancers. One example was the discovery of the BRCA1 gene, mutations of which cause a nearly 100% breast cancer risk in their female carriers [43]. Another case is the mutant APC gene, conferring an increased susceptibility to colorectal carcinoma in some families. Predisposing germline mutations are judged to contribute no more than 5% of all cancers [10]. There is also evidence that germline mutations (such as a point mutation in the Rb1 tumour suppressor gene or in the patched gene) may also make the carrier more radiosensitive with regard to radiation carcinogenesis [44]. The number of people with a severe form of inherited radiosensitivity is probably small, since familial clustering of tumours or patients with multiple tumours in exposed cohorts have not been reported. Also, there are good reasons to assume that susceptibility to radiation-induced carcinogenesis cannot be answered in a yes-or-no manner, but must rather be thought of as a continuously varying feature due to the segregation of multiple predisposing genes [45].

Chakraborty and Sankaranarayanan [46] analysed the problem using the methods of population genetics. Based on the assumption that individuals genetically predisposed to cancer may also be more sensitive to cancers induced by ionizing radiations than those who are not predisposed, they developed a Mendelian autosomal one-locus, two-allele model and demonstrated that “when such heterogeneity with respect to cancer predisposition and radiosensitivity is present in the population, irradiation results in a greater increase in the frequency of induced cancers than when it is absent; this increase is detectable only when the proportion of cancers due to genetic predisposition is large (which it is not) and the degree of predisposition is considerable (which we do not know). Yet even when the effect is small, most of the radiation-induced cancers occur in the predisposed individuals”.

ICRP [10] reviewed the molecular and epidemiological evidence for cancer predisposition and increased radiosensitivity of sensitive subgroups of the general population. The most certain manifestation of increased carcinogenic radiosensitivity would occur in the case of germline mutations associated with inherited deficiency in tumour suppressor genes. This suggestion is supported by observations on second cancers after treatment with radiotherapy of patients affected with bilateral retinoblastoma, nevoid basal cell carcinoma, Li-Fraumeni syndrome and neurofibromatosis. These data suggest a genetically imposed risk increased by a factor of about 10.

Using the methods of population genetics modelling it was concluded that irradiation of a heterogeneous population results in higher cancer risks compared to a population which does not contain radiosensitive subpopulations. However, according to our current knowledge of mutant gene frequencies in the general population relat-

ing to cancer predisposition, no significant distortions of risk estimates would be expected in the heterogeneous population exposed to low radiation doses. There is no indication that after irradiation the risk of excess cancers would be concentrated in a genetically predisposed subgroup of the general population and that the remainder would be relatively radioresistant.

For individuals who carry a predisposing mutation such as BRCA1, ICRP [10] performed some model calculations on radiation-associated increases of risk. It has to be stressed that the increased radiosensitivity is associated with a much elevated risk of spontaneous cancer. Assuming for a genetically predisposed woman who carries the BRCA1 mutation a lifetime risk of fatal breast cancer of about 40% and a tenfold increase in radiation risk of lifetime fatal breast cancer compared to a normal woman of the same age, ICRP [10] estimated that, following a protracted dose of 100 mSv (taken to represent an accumulated occupational exposure), the hypothetical risk of fatal breast cancer in that individual would rise from 40% to 40.4%, as a result of radiation exposure. ICRP [10] concluded that, on this basis, genetic testing for cancer predisposition which has been suggested as a means to improve radiation protection will not play a significant role in occupational exposure to radiation in the future.

Conclusion

Radiation workers and the general public are exposed to ionizing radiations from very different sources. We cannot at present positively exclude the possibility that some members of these populations may develop leukaemia or cancer as a result of these low-intensity radiation exposures. Mathematical models which have been used to estimate the risk by extrapolating from very different exposure scenarios do not take into account the enormous complexity of the carcinogenic process, which only recently is being explored. The linear non-threshold dose response hypothesis may be used as a simple, convenient method to calculate numbers in radiation protection planning but should not be mistaken as a stringent scientific conclusion directly derived from the present state of knowledge of the processes involved in radiation carcinogenesis.

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