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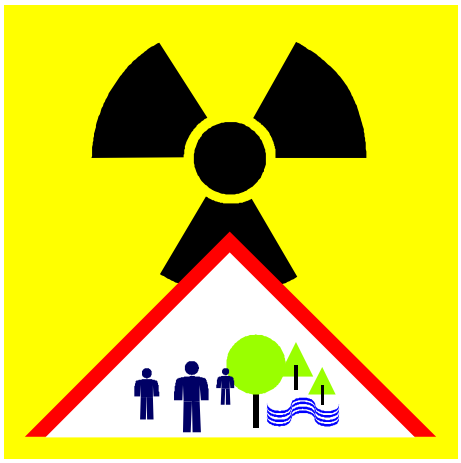

GENETIC SUSCEPTIBILITY AND NEW EVOLUTIONS ON GENETIC RISK

Proceedings of the scientific seminar held in Luxembourg on 29 November 1999

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FOREWORD

It is estimated that about 5% of all cancers are related to predisposing germline mutations. There is evidence that certain germline mutations may also make the carrier more sensitive to ionising radiation and subsequent carcinogenesis. However, it is reasonable to assume that susceptibility to radiation-induced carcinogenesis behaves as a continuously varying feature due to segregation of multiple predisposing genes. Cancer and genetic research to better understand this issue are ongoing.

Under the terms of the Treaty establishing the European Atomic Energy Community, the Community shall, amongst other things, establish uniform safety standards to protect the health of workers and of the general public against the dangers arriving from ionising radiation. The most recent version of such standards is contained in Council Directive 96/29/Euratom of 13 May 1996 laying down basic safety standards for the protection of the health of the workers and the general public against the dangers arising from ionising radiation. The standards are approved by the Council, on a proposal from the Commission, established taking into account the opinion of the Group of experts referred to in Article 31 of the Treaty.

The European Commission organised the seminar on "Genetic Susceptibility and New Evolutions in Genetic Risks in relation to Ionising Radiation" in response to a wish of the members of the Group of experts referred to in Article 31 of the Euratom Treaty to discuss in depth this particular aspect of radiation protection.

The aim of the seminar was to present elements for assessing whether the above-mentioned Directive continues to ensure an adequate level of protection to all citizens of the European Union, irrespective of their individual genetic characteristics at the light of the information resulting from recent scientific research.

Leading scientists in this area, participating in the fourth and fifth European Research Framework Programmes in Radiation Protection, presented the latest developments on the subject.

EMERGING PERSPECTIVES IN RADIATION GENETIC RISK ESTIMATION

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INTRODUCTION

The question of genetic risks of radiation came to the forefront of attention in the aftermath of World War II when nuclear weapons were developed and employed over Hiroshima and Nagasaki. Since then, the field of radiation genetic risk estimation and risk estimates themselves have evolved and several important advances have been made. Up to about the mid-1980s, this evolution was driven primarily by progress in mammalian radiation mutagenesis studies, especially the mouse studies, with much less impact from that in human genetics. Starting in the early 1990s, the situation began to change with the incorporation of insights emerging from human genetics, especially human molecular genetics into the conceptual framework of risk estimation. The major advances that have been made have been periodically reviewed in the various reports published by UNSCEAR, the United Nations Scientific Committee on the Effects of Atomic Radiation, the BEIR Committees of the United States National Academy of Sciences and ICRP, the International Commission on Radiological Protection [e.g., refs. 1-3].

There is no need to belabour the facts that (i) naturally-occurring gene mutations and chromosomal aberrations, if they occur in the germ cells of individuals, can cause genetic diseases in the progeny and that (ii) ionizing radiation is capable of inducing similar types of changes in all organisms investigated in this regard. Putting these two facts together, early on, it was concluded that exposure to radiation can cause an increase in the frequency of genetic diseases in the population. Consequently, the goal of genetic risk estimation, at least as defined and practised by the scientific organizations mentioned above is to estimate the possible increase in the incidence of genetic diseases as a result of radiation exposures to human germ cells.

1. NATURALLY-OCCURRING GENETIC DISEASES AND ESTIMATES OF THEIR FREQUENCIES

1.1. Genetic diseases and their classification

Genetic diseases are those that arise as a result of mutations in germ cells and which are transmitted to the progeny. Humans carry 22 pairs of ordinary chromosomes called autosomes and a pair of sex-chromosomes called the X and Y. Males have 22 pairs of autosomes and an XY pair while females have 22 pairs of autosomes plus 2 X chromosomes. Mutations can arise in genes contained in the autosomes or the sex chromosomes of either ordinary body cells or of the germ cells of parents (contained in the testes/ovary). Those arising in ordinary body cells can cause cancer in the individual

but are not transmitted to the next generation. Those arising in germ cells are transmitted to the next and following generations and are therefore called hereditary or genetic diseases.

Depending on transmission patterns, these diseases can be divided into mendelian and multifactorial diseases. Mendelian diseases are due to mutations in single genes and are further subdivided into autosomal dominant, autosomal recessive and X-linked ones. The Y chromosome does not contain many genes other than those involved in sex determination. In the case of an autosomal dominant disease, a mutation in an autosomal gene of either the father or the mother is sufficient to cause disease in the child. Examples include neurofibromatosis, myotonic dystrophy and polycystic kidney disease. In the case of an autosomal recessive disease, two mutations of the same autosomal gene, one from each parent is necessary for disease causation, as can happen when close relatives marry and have children. Examples include cystic fibrosis, homocysteinuria and a number of enzyme deficiencies. X-linked diseases are due to mutations in genes on the X-chromosome. Since males have only one X chromosome, only males are usually affected. Examples include Duchenne muscular dystrophy, fragile X syndrome and hemophilia. The important point with respect to mendelian diseases is that the relationship between mutation and disease is straightforward and the pattern of transmission is simple and predictable.

Multifactorial diseases are those which arise as a result of the joint action of multiple genetic and environmental factors. These factors can vary between individuals, racial groups and populations. Examples include the common congenital abnormalities (e.g., neural tube defects, congenital heart defects, cleft lip with or without cleft palate etc) which are present at birth and chronic diseases of adults such as diabetes, essential hypertension, coronary heart disease etc. These diseases do not show simple patterns of inheritance i.e., the relationship between mutation and disease is complex. However, these diseases do run in families.

1.2. Baseline frequencies

Recent estimates suggest that about 2.4% of all liveborn children suffer from one or another mendelian disease (1.5% autosomal dominants, 0.75%, autosomal recessives and 0.15%, X-linked) [4]. Additionally, about 6% of livebirths are affected by one or another congenital abnormality and over 65% of the population will develop one or another chronic disease in adult life [1].

2. RISK ESTIMATION

Due to the paucity of human data on radiation-induced adverse genetic effects, already in the 1950s geneticists chose the mouse as an appropriate model to assess these effects in humans, a practice that has continued to the present. In the mouse studies, the emphasis has been on obtaining data on radiation-induced mutations, their frequencies and rates. The genes at which induced mutations were studied were actually not chosen from the disease point of view, but rather by their sensitivity to induced mutations (i.e., obtaining mutant individuals in reasonably large numbers) and the ease with they can be identified. Consequently, methods had to be developed to convert the data on induced mutations in mice into the risk of genetic disease in humans. By necessity, they are all indirect methods and such extrapolation from mouse to humans involve uncertainties.

2.1. The doubling dose method

One such indirect method, which is still in current use, is called the "Doubling dose method" and is based on the population genetic theory. The concept is that that the relatively stable incidence of genetic diseases in a population is a reflection of the existence of a balance between mutations that arise spontaneously in every generation and natural selection which eliminates some of these mutations in every generation through death or failure of reproduction. When the population is exposed to radiation, say, in every generation, the population will eventually strike a new balance between mutation and selection, at a higher mutant, and thus of disease frequencies.

With the DD method, the risk is estimated as a product of three quantities, namely, P, 1/DD and MC as summarized in equation (1) below:

$$\text{Risk per unit dose} = P \times [1/DD] \times MC \qquad (1)$$

in which P is the natural incidence of the disease class under study before radiation exposure, DD refers to the doubling dose and MC is the "mutation component". The doubling dose (DD) is the amount of radiation required to produce as many mutations as those that occur spontaneously (i.e., in the absence of radiation) in a generation. It is estimated by dividing the average spontaneous mutation rate of a set of genes by the average rate of induced mutations in the same set of genes. The reciprocal of the DD is the relative mutation risk (RMR) per unit dose and Gy is the unit of radiation dose (= 100 rads in the old system). As can be readily noted, a low DD implies high RMR and vice versa: if for example, the DD is 1 Gy, the RMR = 1 if the DD is 4 Gy, then the RMR is 1/4. The DD so far used in risk estimation is 1 Gy and is based predominantly on mouse data on recessive mutations in 7 genes, which have been extensively studied.

2.2. Revision of the conceptual basis and magnitude of the doubling dose

Recently, the use of an entirely mouse-data-based DD for estimating the risk of genetic disease in humans and the numerical value of 1 Gy have been questioned and re-examined in detail [4]. This has led to the introduction of both a conceptual and a numerical change in calculating the DD. The conceptual change pertains to the use of human data on spontaneous mutations and mouse data on induced mutations for calculating the DD. The numerical change is the revision of the DD from the 1 Gy thus far used to 1.5 Gy. This revision is based on an extensive analysis and use of the estimates of the average rate of spontaneous mutations in human genes and the average rate of induced mutations in all mouse genes for which data are available [K. Sankaranarayanan et al., unpublished].

2.3. Mutation component

The third quantity in the risk equation (1) mentioned earlier is what is referred to as the mutation component (MC). It provides a measure of how the disease frequencies will change when the mutation rate is changed, as for example with radiation exposures. The reason for having this quantity in the risk equation is that the relationship between mutation and disease varies between different classes of genetic diseases. The estimation procedure is simple for autosomal dominant and X-linked diseases, slightly complex for autosomal recessive diseases and very complex for multifactorial diseases.

Although the mutation component concept was originally enunciated by Crow and Denniston in 1981 [5], it is only during the last 3 to 4 years, we were able to develop the MC concept fully for mendelian and multifactorial diseases. This was done within the framework of an ICRP Task group [6]. We can now estimate the MC for any post-radiation generation of interest for any of the above three classes of diseases. For example, for the first generation following radiation exposure, the MC can now be estimated to be of the order of about 0.3 for autosomal dominant and X-linked diseases, close to zero for autosomal recessive diseases and 0.01 to 0.02 for multifactorial diseases [7, 8].

It should be noted that for estimating MC, the standard population genetic models based on the equilibrium theory were used for mendelian diseases [7] and that a modified multifactorial threshold model referred to here as the Finite Locus Threshold Model (FLTM) was used for multifactorial diseases [8]. A key assumption of the FLTM is that mutations are induced in all the genes underlying a multifactorial disease (up to five genes were assumed). While this assumption is biologically unrealistic at low radiation doses, the important point is that even with this "maximal" assumption, the estimated MCs for the first few generations (under conditions of radiation exposure in every generation) were small (in the range of 0.01 to 0.02). Stated differently, the MC estimates derived by using the FLTM are overestimates for the first few generations.

3. GENETIC STUDIES CARRIED OUT ON THE CHILDREN OF A-BOMB SURVIVORS IN JAPAN

In these studies [9], the emphasis had always been to obtain a direct measure of the magnitude of adverse consequences of gonadal radiation exposures, using indicators of damage that were practicable at that time; they were not aimed at expressing risks in terms of what would be formally called genetic diseases. This is in contrast to the approach used by UNSCEAR and the BEIR committees: these scientific bodies have been looking at genetic risks of radiation through the "prism" of naturally-occurring genetic diseases. I will return to the consequences of this difference in approach later.

The principal indicators of genetic damage used in the Japanese studies, were (i) untoward pregnancy outcomes (UPOs which included stillbirths, early neonatal deaths and congenital abnormalities in livebirths); (ii) survival of children through their mid-20s; (iii) malignancies; (iv) balanced chromosomal rearrangements (v) sex-chromosomal aneuploids; (vi) mutations affecting protein charge or function (vii) growth and development of infants and (vi) sex-ratio shifts.

The main conclusions from these studies -- the largest ever undertaken -- are (i) there was no demonstrable difference in any of the measures used and (ii) the data were statistically consistent with a DD as high as about 4-5 Gy. Recall that a high DD means low relative mutation risk. These findings therefore support the view that the risks are perhaps lower than implied by the earlier mouse-based DD of 1 Gy and the now revised DD of 1.5 Gy based on human spontaneous and mouse induced rates of mutations. As discussed below, it is now possible to resolve the discrepancy between these DD estimates.

4. THE CONCEPT OF POTENTIAL RECOVERABILITY CORRECTION FACTOR (PRCF) AND THE DOUBLING DOSE ESTIMATES

When one uses the risk equation Risk = P x 1/DD and MC, it should be realized that it is a predictive equation based on population genetic theory. The quantity P defines what societally important diseases radiation may induce, and the product of P, 1/DD and MC defines the magnitude of such induced diseases. However, no one has seen a single radiation-induced genetic disease so far in humans. Advances in human molecular biology and molecular analysis of radiation-induced mutations support the view that spontaneous disease-causing mutations in humans and radiation-induced mutations studied in experimental systems differ in a number of ways and consequently, the assumption that radiation-inducible genetic diseases would be similar to the naturally-occurring ones is incorrect [10].

First, the molecular changes identified in genetic diseases include point mutations, small and large intragenic deletions and some multigene deletions. However, although all these changes are produced by radiation, most radiation-induced mutations are multigene deletions. Second, the different types of changes seen in spontaneous mutations arise through a variety of mechanisms. Most of these mechanisms are dependent on the DNA sequence organization of genes and their genomic context. Radiation, however, produces mutations by random deposition of energy.

So, one can assume that the initial probability of damage induction is the same for all regions of the genome. But whether that induced mutation, say, a deletion, will be seen in the progeny of an irradiated parent depends on its effects on survival of the progeny receiving it. One would expect that, in some genes, radiation-induced mutations will be recovered at high frequencies because that gene and/or genomic region containing it is non-essential for survival whereas some genes will respond with low frequencies or not at all because that gene and/or genomic region cannot sustain recoverable deletions (i.e., those compatible with viability of the offspring).

Third, the effects of mutational changes seen in naturally-occurring genetic diseases include those which result in loss of function of genes as well as those that cause gain of function. Radiation-induced mutations in contrast, are predominantly of the loss of function type, because they are multigene deletions.

One is tempted to ask: with all these differences, how come we have been very successful in inducing mutations in experimental systems? The answer is simple: most of the genes chosen for studies of induced mutations in mice and other organisms are not essential for survival of the individual and also happen to be located in genomic regions that are not essential for survival. However, most of the human genes of interest from the disease standpoint are not of this type. The inference, therefore, is that only a small proportion of genes in our genome are potentially capable of responding to induced mutations compatible with survival and hence potentially recoverable in livebirths.

Consequently, there is a need to introduce a correction factor to bridge the gap between the rates of induced mutations that are recovered in mice and those of induced mutations that are potentially recoverable in humans. We call this correction factor, the “Potential Recoverability Correction factor” [K. Sankaranarayanan and R. Chakraborty, unpublished] and suggest its inclusion in the risk equation so that the latter now includes four factors instead of the original three:

$$\text{Risk} = P \times [1/DD] \times MC \times PRCF \qquad (2)$$

Such a correction factor can be derived by first developing a set of criteria based on mutations recovered and studied extensively in the mouse, their genes, sizes, function, genomic context etc., and applying these criteria to human genes of interest on a gene-by-gene basis taking to account all that is known about them. The question asked is: if a deletion were to be induced in this region, is it recoverable in a livebirth? Such an inquiry was carried out for a total of 63 human genes which when mutated cause autosomal dominant and X-linked diseases. The analysis revealed that only about 21 of the 63 genes or roughly 30% could be considered responsive to potentially recoverable induced mutations. If weighted by their respective incidences, this proportion is even smaller, namely 15%. In other words, the PRCF for autosomal dominant and X-linked diseases can be considered to be in the range of 0.15 to 0.30. For autosomal recessive diseases, such calculations are unnecessary since for these diseases, one of the factors in the risk equation, i.e., MC, is close to zero.

For chronic multifactorial diseases, however, the situation is different. Recall that these diseases arise as a result of interaction between multiple genetic and environmental factors. In the radiation context, with the model used, one assumes that mutations are induced simultaneously in all the underlying genes. If, as mentioned earlier, the PRCF for single gene diseases is in the range of 0.15 to 0.30, for chronic multifactorials, the PRCF should be 0.15 to 0.30 raised to the power *n* where *n* is the number of genes underlying the disease. Even if there were only two genes, the PRCF range becomes $(0.15)^2$ to $(0.30)^2$ or 0.02 to 0.09. It is obvious that when more genes are involved, the PRCFs will be very small indeed.

As will be evident, with the introduction of PRCF into the risk equation, the discrepancy between the Japanese DD of 4 to 5 Gy and the DDs of 1 Gy (mouse-data-based) and 1.5 Gy (the revised DD) can be reconciled: in the Japanese studies, the DDs are *retrospectively* estimated from empirical observations in humans and therefore, there is no need to introduce PRCFs. In the case of the other DDs (which are *prospectively* applied to predict risk), this is not the case and so there is a need to introduce PRCF in the risk equation to take into account recoverability of radiation-induced mutations. Consequently, the DD estimated from the Japanese data and the other two cited above are not comparable. One would expect that the DD of 1 Gy or 1.5 Gy divided by the PRCFs should yield estimates similar to those obtained in Japanese studies which is indeed the case (e.g., 1.5/0.3 = 5 Gy).

5. POTENTIAL PHENOTYPES OF RADIATION-INDUCED GENETIC DAMAGE IN HUMANS

In the discussion so far, it has been assumed that ionizing radiation will induce mutations in specific genes resulting in specific genetic diseases that we are interested in. However, as is well known, radiation produces mutations by random deposition of energy and it does not "know" that the risk estimators are interested in societally relevant mendelian and multifactorial diseases. It will produce damage somewhere in the genome, mostly deletions. Whether such induced deletions will be recoverable in livebirths and what their phenotypes are likely to be, depend on what gene functions have been lost.

Some insights into the potential phenotypes of radiation-induced multigene deletions come from studies of the so-called microdeletion syndromes in humans. These are deletions of multiple, functionally unrelated yet physically contiguous genes that are

compatible with survival in the individuals receiving them. Several examples of microdeletion syndromes have been reported in the human genetics literature. They show that their distribution in different chromosomes is non-random. This is not unexpected in the light of differences in gene density in different chromosomes and chromosomal regions. However, the important point is that despite their occurrence in different chromosomes, they share some common features: mental retardation, growth retardation, specific patterns of dysmorphic features, serious malformations etc. This is because of the fact that genes involved in developmental processes are enormous in number and are distributed in nearly all the chromosomes.

It has therefore been suggested that the principal type of adverse genetic effects of radiation will be manifest as multisystem developmental abnormalities, which we call congenital abnormalities [10]. Their phenotypes are not as clean or clear-cut as those of single gene diseases. In other words*, the principal genetic risk of radiation is not from induced single gene diseases of the types listed in McKusick's catalogue, but these developmental defects*. Since most of the induced developmental abnormalities are due to multigene deletions, they would be expected to show autosomal dominant pattern of inheritance in contrast to most of the naturally-occurring ones that are interpreted as being multifactorial.

There are some mouse radiation data on congenital malformations, growth retardation, dominant skeletal defects and dominant cataracts in the progeny of irradiated mice. From all these, one can estimate that the rate is of the order of about 30 x 10^{-4}/gamete/Gy for acute irradiation and about 10 x 10^{-4}/gamete/Gy under chronic radiation conditions. Note that one does not need to use the DD method here. The closest comparison with the human data would be with 26.4 x 10^{-4}/zygote/Sv calculated by Neel for UPOs in the Japanese studies.

6. CURRENT RISK ESTIMATES

Table 1 presents a summary of the current risk estimates (revised subsequent to those in the 1993 UNSCEAR report) for the first generation progeny of an irradiated population [K. Sankaranarayanan et al., unpublished]. I should stress that at present, these estimates are "personal" i.e., they have not yet been approved by international committees involved in the assessment of radiation risks. These will be presented, however, to UNSCEAR for discussion at its forthcoming meeting in May 2000.

Table 1. Current estimates of genetic risks of low LET, low dose-rate irradiation to the first generation progeny (The doubling dose assumed in these calculations is 1.5 Gy)

Disease class	Natural Incidence per million livebirths	Risk per Gy in a million livebirths
Autosomal dominant and X-linked	16,500	500-1000
Autosomal recessives	7,500	~ 0
Chronic multifactorial	650,000	200- 800
Congenital abnormalities	60,000	~1000

Inspection of Table 1 will show that the risk of autosomal dominant and X-linked diseases is of the order of 500-1000 cases per million progeny per Gy of chronic radiation (compared to 16,500 cases per million of naturally-occurring ones). The risk of autosomal recessive diseases is essentially zero (compared to 7500 per million naturally-occurring ones). The risk of chronic diseases is of the order of 200 to 800 cases per million per Gy (compared to 650,000 per million naturally-occurring-ones). The risk of multisystem developmental abnormalities may be of the order of about 1000 cases per million per Gy.

7. POTENTIAL IMPACT

First, all the material that I discussed is being incorporated into the draft of the Genetics annex of the forthcoming UNSCEAR report scheduled for the last round of discussions in May 2000. If the scientific basis of these risk estimates and the estimates themselves are accepted without much modifications, then that will be an important step towards their possible consideration in the BEIR VII report expected to be published in the year 2002 or so and also by ICRP in the revision of Publication 60 which is contemplated in the next five years or so.

8. SUMMARY

This paper reviews the recent advances in the field of genetic risk estimation and present revised risk estimates (i.e., subsequent to those presented in the 1993 UNSCEAR report). The advances include: (i) the updating of the baseline frequencies of mendelian diseases; (ii) the revision of the conceptual basis and magnitude of the doubling dose; (iii) development of methods to estimate MC for mendelian and multifactorial diseases; (iv) development of the PRCF concept; and (v) delineation of the principal phenotypes of radiation induced genetic damage in humans. Of these, items (iv) and (v) incorporate advances in human molecular biology. As a result of these developments, it has now become possible, for the first time in over 40 years, to provide risk estimates for all classes of genetic diseases.

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MOLECULAR MECHANISMS OF IONIZING RADIATION-INDUCED DNA DAMAGE REPAIR

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1. CELLULAR RESPONSES TO DNA DAMAGE

The essential information for the proper functioning of all cells in the human body is stored in DNA. If unfolded and placed end-to-end, the DNA molecules in each cell span two meters in length. Endogenous and exogenous DNA-damaging agents are constantly challenging the integrity of this central information carrier. To minimize the harmful effect of DNA damage, a number of protective responses have evolved, including cell cycle checkpoints and DNA repair. The induction of DNA damage results in activation of checkpoints that cause dividing cells to pause to allow time for DNA-damage repair. Inherited disorders associated with defects in cell cycle checkpoint activation, such as ataxia telangiectasia and Nijmegen breakage syndrome [1], reveal the importance of checkpoints. These disorders cause hypersensitivity to DNA-damaging agents and spontaneous chromosomal instability. The significance of DNA repair is illustrated by the phenotypes of xeroderma pigmentosum, Cockayne's syndrome, trichothiodystrophy and hereditary nonpolyposis colorectal cancer patients [2,3]. These disorders are caused by mutations in DNA repair genes that predispose the patients to cancer and/or neurological abnormalities.

2. PATHWAYS OF DSB REPAIR

Ionizing radiation is useful in killing proliferating cells during anti-cancer therapy because it introduces double-strand breaks (DSBs) into the DNA. In addition, endogenous DNA damaging agents, such as reactive oxygen species generated by oxidative metabolism can cause DSBs. Repair of these endogenously generated DSBs is important for the prevention of genomic instability that could lead to carcinogenesis. The deleterious effects of DSBs have resulted in the evolution of multiple DSB repair pathways [4]. Understanding their mechanism is important because they counteract the therapeutic effect of ionizing radiation. Two major DSB repair pathways are homologous recombination and DNA end joining. Homologous recombination requires extensive regions of DNA homology and repairs DSBs accurately by using information on the undamaged sister chromatid or homologous chromosome. In contrast, DNA end joining uses no or extremely limited sequence homology to rejoin ends in a manner that need not be error free.

3. MOLECULAR MECHANISMS OF DSB REPAIR

To analyze the molecular mechanisms of DSB repair it is important to identify the genes that mediate this process. These genes are essential for the generation of tools that allow analyses of the proteins involved in DSB repair with the use of genetic, cell biological, and biochemical techniques. A set of genes, called the *RAD52* epistasis group of genes,

has been shown to be required for DSB repair through homologous recombination in the baker's yeast *Saccharomyces cerevisiae* [4]. This group includes the *RAD51* and *RAD54* genes (see Figure). Subsequent experiments revealed that the *RAD52* group genes are also present in mammalian cells and that the Rad51 protein is a central player in homologous recombination because it mediates the search for homologous DNA [4,5].

DSB repair through DNA end joining has been analyzed using ionizing radiation-sensitive Chinese hamster ovary cell lines [6]. A multi-protein complex that is involved in DNA end joining is the Rad 50/Mre 11/Nbs 1 complex [7]. Biochemical experiments have implicated the Mre 11 protein in processing of the DNA ends before repair. In addition to its role in DNA repair, the Rad 50/Mre 11/Nbs 1 complex is likely to play a role in activating cell cycle checkpoints upon the induction of DNA damage, because it was shown recently that the *NBSI* gene is mutated in Nijmegen breakage syndrome patients [8].

4. NUCLEAR DYNAMICS OF DSB REPAIR

DSBs must be repaired irrespective of their position within the cell nucleus. Therefore, it might be expected that the proteins involved in DSB repair could undergo dynamic changes in their position within the nucleus upon the induction of DSBs. Recent immunofluorescence experiments have provided evidence for such a dynamic behaviour. These experiments show that upon treatment of cells with ionizing radiation, the Rad51 protein relocates into bright nuclear foci (see Figure) possibly at the sites of DNA repair [9,10]. DNA damaged-induced foci formation also occurs within the DNA end joining pathway (see Figure). The relocalization of the Rad 50/Mre 11/Nbs 1 complex is important for DNA repair because upon irradiation of part of the nucleus of human fibroblasts, Rad 50/Mre 11/Nbs 1 foci occur only in the irradiated volume [11].

5. FUTURE PERSPECTIVES

Gaining additional understanding of the molecular mechanisms of ionizing radiation-induced DNA damage repair is pivotal in light of the common use of ionizing radiation in the treatment of both malignant and benign diseases. A large number of mammalian genes involved in the repair of ionizing radiation-induced DNA damage has been identified in recent years. These genes provide the tools for probing the mechanism of the DNA repair processes. Therefore, it is expected that considerable progress can be made in the coming years. For example, the finding that proteins involved in different DSB repair pathways form ionizing radiation-induced foci that are detectable by immunofluorescence in analyzing the effects and efficacy of radiotherapy.

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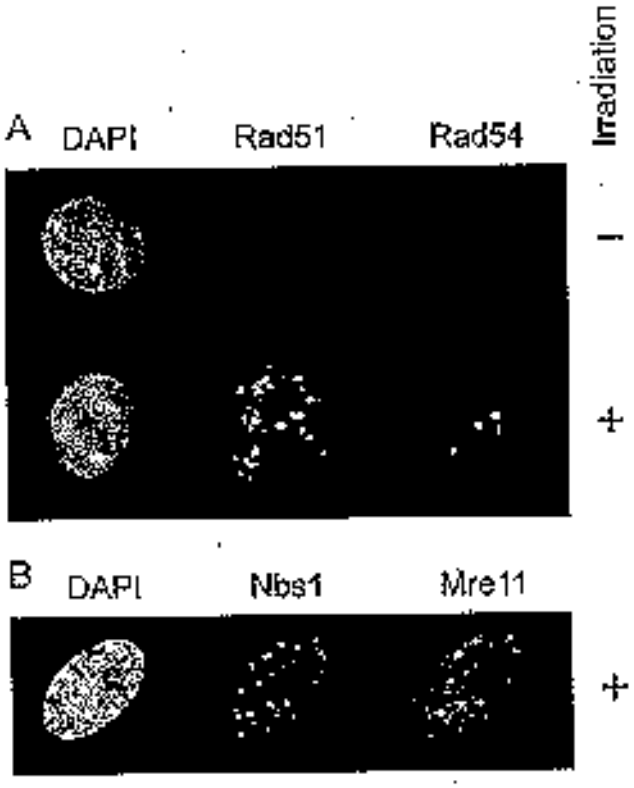
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RADIATION INDUCED CHROMOSOMAL INSTABILITY

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Radiation exposure will induce many DNA damage and most of them will be repaired or processed in the minutes or hours following irradiation. Then the lesions will be considered "frozen" and will be transmitted or not to the progeny. But, over the years, there are evidences that de novo instability can manifest many cell generations after cellular irradiation leading to the accumulation of damages in the surviving cells. In human fibroblasts irradiated by heavy ions in a large range of LETs, we showed that the chromosomal instability arising 15 passages after irradiation is characterized by telomeric associations (TAS) involving specific chromosomes (Sabatier *et al.* 1992, Martins *et al.* 1993). Transmissible chromosomal instability was detected after alpha-Pu-238 irradiation in murine and human hematopoietic cells (Kadhim *et al.* 1992, Kadhim *et al.* 1994). In different cellular models of irradiation of primary cells, chromosomal instability was mainly detected after heavy ion irradiation. However, clonal rearrangements and chromosomal instability were identified after X-ray irradiation of human lymphocytes (Holmberg *et al.* 1993, Holmberg *et al.* 1995). These data reinforced the pioneer experiments of J. Little's laboratory showing accumulation of damage in the progeny of irradiated cells: gene mutation, cell survival cellular transformation, microsatellite instability (Kennedy *et al.* 1980, Kennedy *et al.* 1984, Chang and Little 1991, Chang and Little 1994, Li *et al.* 1994). The dose-response relationship of delayed damage was tested in V79 cells showing a steep increase of the frequency with dose up to 3-4 Gy and no further increase at higher irradiation (Jamali and Trott 1996). However, chromosomal instability might be more linked to the characteristic of the donor rather than to the dose as well in vitro (Kadhim *et al.* 1998) as in vivo (Watson, 1996; Ponnaiya, 1997). The de novo instability might be an important step in the understanding of biological effect of ionizing radiations such as delayed cell death or cell transformation (Sinclair 1964). But the mechanisms underlying this phenomenon are still unknown. A major reason could be that de novo chromosomal instability is an equivocal concept. Different types of chromosome damages occur de novo in the long-term progeny of irradiated cells.

It has been described - structural rearrangements : chromatid type (post- replicative), non clonal chromosome type, clonal, specific or not of some structures or chromosome

- aneuploidies : gain or loss of chromosomes (arms)

However, different biological endpoints would have different consequences : chromatid type would lead preferentially to cell death otherwise, chromosome type would be more often transmitted to cell progeny.

Chromosome modifications recensed in human tumors cells could be classified in four cytogenetic groups :

- *Monosomic Type* is characterized by high frequency of rearrangements (deletions) and chromosome losses leading to hypodiploidy or hypotetraploidy (down to 50) after endoreduplication. In most cases there is a direct association with loss of function

(RB1, P53, P16, APC...) This type is the most frequent in epithelial cancers (Breast : majority, lung : all? colorectal : 70%)

- *Trisomic Type* results from a progressive increase of the number of chromosomes towards a pseudo-triploidy, with only few rearrangements, and rare deletions. There is no hypothesis on the molecular background, except for a gene dosage effect. This type is observed in endometrium adenocarcinomas, colorectal adenocarcinomas (25%) and is frequent in non epithelial cancers: half cases of neuroblastomas, Wilm's tumors ...

- *Translocation Type*: the presence of a balanced rearrangement is detected, most frequently a translocation leading to the formation of a fusion (onco-) gene, or activation of a proto-oncogene (dominant). This type is exceptional in epithelial cancers and frequent in sarcomas. The karyotype remains pseudo-diploid, with very few additional rearrangements, some gains and very rare deletions.

- *« Normal »* Type: This type is probably rare, but frequence unknown. It is described in colorectal cancer 5 - 7% of cases (right colon, HNPCC patients).

The direct link between directly radiation-induced chromosome damage and the cytogenetic modifications detected in human cells is highly unprobable. However the chromosome imbalances detected in long term progeny of irradiated cells could play the key role in the occurrence of abnormal karyotypes. The specific chromosomal instability that we observed after irradiation of human fibroblasts is characterized by end-to-end associations. It would not be a direct consequence of irradiation but would be a natural phenomenon occurring after many cell divisions. The effect of the irradiation would lie on the bypass of the senescence process which would permit cells with end to end fusions to survive and be transmitted through cell generations, accumulating chromosome rearrangements and chromosome imbalances (Pommier et al in prep). Irradiated cells would have performed some steps towards cell transformation

There is a correlation between the ability of chromosomal instability induction and cancer predisposition (in mice). Chromosomal instability is linked to radiation and to general cancer development. Irradiation induces the early occurrence of chromosomal instability. However, the induction of chromosome damages by irradiation in radiation-induced tumours is not a direct causal event. Chromosomal instability occurring in the progeny of irradiated cells will generate new chromosome rearrangements leading to chromosome imbalances (secondary events) and will unmask recessive mutations constitutional or radiation-induced.

The mechanisms of induction are unknown and could differ depending of the different kind of instability detected :

- « passive » or « active » induction

1) passive : irradiation induces recessive mutations, irradiation induces cell death, surviving cells can proliferate, « premature aging » chromosomal instability occurs during the beginning of senescence process, cells will not senesce if some unmasked recessive mutations confer a proliferative ability

2) active: radiosensitivity of specific chromosome structures (ie telomeres : fragility, repair efficiency?) or epigenetic factors (ie methylation) -> gene expression regulation or radical species (clastogenic effect, cell death, chromatid breaks...)

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GENETIC SUSCEPTIBILITY TO CANCER

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INTRODUCTION

It has been known for many years that within the human population there are individuals and families who carry heritable susceptibility to spontaneously arising cancer. The first mechanistic link between heritable cancer and an environmental carcinogen was forged in the late 1960s when it was demonstrated that excess skin cancer in sun-exposed xeroderma pigmentosum (XP) patients was due to a defect in the repair of DNA photo products. Since the 1960s there has been very rapid development in the whole area of cancer genetics fuelled by advances in medical genetics and cell/molecular biology[1]. These advances have led to the identification and characterisation of a range of cancer-associated disorders and evidence on the importance of DNA damage response and tumour suppressor gene deficiencies. This work has included the identification of DNA repair-deficient disorders, ataxia-telangiectasia (A-T) and Nijmegen breakage syndrome (NBS), that show profound increases in sensitivity to ionising radiation. Around 10% of known Mendelian human genetic disorders show some association with cancer and for perhaps 100 such disorders the evidence is unambiguous.

In the early 1990s the International Commission on Radiological Protection (ICRP) was giving careful thought to the possible implications of such cancer predisposition for radiological protection. An ICRP Task Group was formed in 1993 with a brief to review the field and to develop a scientific framework on which to begin to address questions regarding the possible impact on population risk after radiation, effects in individuals and the attendant issue of genetic testing. Following review, revision and adoption the Task Group Report was published in 1998[2] and a similar review conducted by an advisory group to the UK National Radiological Protection Board (NRPB) has been published recently[3]. These documents provide the source material and supporting references for the principal judgements outlined in the present paper.

1. CANCER PREDISPOSITION AND RADIOSENSITIVITY

Although at first sight somewhat counter-intuitive, there is not a simple relationship between genetically determined cellular radiosensitivity and cancer predisposition. In some human autosomal recessive genetic disorders of DNA damage response, radiosensitivity in respect of cell killing and/or chromosomal damage is associated with excess cancer, i.e. A-T and NBS (Table 1). However cancer-prone, autosomal dominant disorders of tumour suppressor genes (Table 2) do not, usually, exhibit cellular radiosensitivity although in some there may be cell cycle-dependent changes in chromosomal response. The mechanistic reason for this is that germ line deficiency in one copy of a given suppressor gene effectively unshields the remaining copy in all somatic cells of the carrier. The life-time risk of spontaneous loss/mutation of this remaining copy from a target cell is high, hence the greatly elevated probability of cancer development. In this way, although the cells of the carrier individual are not overtly

radiosensitive in a conventional fashion, it is fully expected that exposure to DNA damaging agents such as ionising radiation would increase the frequency of this second mutation. Stated simply, a reduction in the target gene number from two (normal) to one (predisposed) will provide for elevated tumorigenic radiosensitivity in these disorders. On this basis the judgement overall is that genetically increased susceptibility to spontaneous tumorigenesis will be accompanied in most, but not all cases, by increased cancer risk after radiation. The principal exceptions to this broad judgement will be a) DNA repair deficiencies like XP where the repair function has little consequence for ionising radiation-induced DNA damage and b) deficiencies in metabolic functions associated with chemical carcinogens which are irrelevant to radiation action.

Given these judgements it is possible to approach further questions on the population prevalence of relevant disorders, organ specificity for tumour development and the likely magnitude of enhancement of tumorigenic radiosensitivity.

2. POPULATION PREVALENCE

Review of available data supports the view that strongly expressing (high penetrance) disorders of cancer are very rare in the population. As given in the Tables these prevalences range from around 1 in 1000 to <1 in 100,000 live births; taken together and even allowing for some underestimation it seems likely that <1% of western populations fall into this genetic category and that they account for around 5-10% of total cancer in these populations. This contribution will however be highly age-dependent since a general characteristic of such disorders is that the age of tumour onset is almost invariably earlier than that in the general population. A major uncertainty in respect of prevalence of cancer-predisposing disorders is associated with problems of tumour ascertainment and size of kindreds for study. Unless tumour records are good and kindred sizes are large it is not possible to identify weakly expressing (low penetrance) genes. This is an important scientific deficiency since there is reason to believe that low penetrance mutations will be more common in the population.

3. ORGAN-SPECIFICITY OF TUMORIGENESIS

There are some human genetic disorders such as Li Fraumeni syndrome (LFS) where tumour predisposition applies to a wide range of organs. Usually, however, there is strong organ specificity for the disorders that have been characterised as being cancer-predisposing. In the case of disorders involving tumour suppressor genes this is believed to be associated with the organ-specific function of these genes. Many such genes have been characterised as ‘gate-keepers’ for maintaining normal growth control of specific cell lineages – when this function is lost a specific tumorigenic pathway becomes open.

In the case of DNA repair deficiency as it applies to DNA strand breakage, there are known implications for the structural rearrangement of immune function genes. This provides a coherent, but probably not complete, explanation of excess lympho-haemopoietic neoplasms in A-T and, perhaps, NBS patients. In cases of hereditary non-polyposis colon cancer (HNPCC) the causal deficiency is associated with DNA mismatch repair.

4. QUANTIFICATION OF ENHANCED TUMOUR RISK

Three principal sources of information have contributed to interim judgements on the degree to which radiation cancer risk in the known and relevant genetic disorders may be elevated over that of the 'normal' population.

Cellular data: Data on cellular radiosensitivity particularly that relating to chromosomal damage can provide some guidance in respect of disorders of DNA damage response. In A-T and NBS some cytogenetic assays suggest elevated radiosensitivity up to ~10 fold but in most of the human disorders so far examined the elevation is more modest (say 2-3 fold).

Animal data: Modern techniques in animal genetics have allowed the identification or genetic construction of rodent mutants that recapitulate specific human cancer prone disorders. Crucially, experimental studies with three such tumour suppressor gene deficient rodents have fully confirmed the elevated tumorigenic radiosensitivity predicted from the mechanistic considerations outlined in section 2. Quantification of the elevated risk has proved somewhat problematical since the genetic background of the host animal can have profound effects; although problematical, such effects are being exploited to gain fundamental information on germ line gene-gene interactions. The data overall for these mutant rodents point towards a range of 10-100 fold increased tumour risk after radiation with the most comprehensive data set for *p53* gene-deficient mice (homologue of human LFS) suggesting a value of 10-15 fold. Although yet to be published in full, there are some data from studies with animal models suggesting that genetically determined tumour risk after exposure to DNA damaging agents is maximal at young ages.

Human data: There are few sources of human information of direct relevance to the problem of tumorigenic radiosensitivity. Of greatest utility are case reports and epidemiological follow up of cancer–predisposed patients receiving radiotherapy (RT) for first tumours. Case reports showing excess basal skin neoplasms and ovarian tumours in the irradiated field of nevoid basal cell carcinoma syndrome patients provides good evidence of substantially elevated and early expressing tumorigenic radiosensitivity; this risk has yet to be quantified. The same applies to excess tumours in follow up of LFS patients receiving RT. Initial follow-up of occular RT in large numbers of heritable and non-heritable retinoblastoma patients, whilst not without problems of interpretation, does allow the interim judgement of a 5-10 fold increased tumour risk in the heritable form. Further follow up of these cohorts did not however resolve uncertainties and served principally to highlight inherent problems regarding quantification of risk.

Equally problematical has been the search for an association between breast cancer risk and heterozygosity for the *ATM* gene of ataxia-telangiectasia. At the cellular level such individuals are marginally radiosensitive but in spite of much molecular epidemiological study the issue of breast cancer risk remains unresolved. These studies do however cast great doubt on early claims of high breast cancer risk in A-T heterozygotes receiving very low radiation doses.

Other relevant human investigations include study of Japanese A-bomb survivors and the ~5% of human RT patients who experience unusually strong early tissue reaction. In the Japanese studies an unexpected excess of early onset breast cancer in the women irradiated before 20 years of age is suggestive of a radiosensitive sub-group. Current data argue against a simple relationship between normal tissue reaction in RT patients and

cellular radiosensitivity. However, the study of RT patients has yet to be extended to investigate possible associations between early tissue reaction and late expressing tumorigenesis.

5. COMPUTATIONAL MODELLING OF CANCER RISK

The ICRP Task Group gave much attention to developing and utilizing population genetic models to describe the possible impact of genetically-predisposed sub-groups on radiation cancer risk in the whole population. For reasons of data availability and reliability, breast cancer risk was modelled using the information and judgements developed by the Group. Input parameters to the model of choice were varied in order to gain a view of a range of scenarios. Particular attention was however given to examining the consequences of a 10 fold increase in risk which was judged to be the risk enhancement value that best described the various data sets that had been considered. At the outset it was recognised that this was a simplistic judgement and the computational modelling was largely illustrative. It was also stated that these judgements could apply only to the strongly expressing human disorders of which there was some knowledge. The implications of this modelling exercise and other practical issues are outlined below.

6. INTERIM JUDGEMENTS ON IMPLICATIONS FOR RADIOLOGICAL PROTECTION

Risks in the whole population: Since current estimates of radiation risk are based upon direct epidemiological study of genetically heterogenous whole populations, any component of genetically determined increased risk is already included. The problem is not therefore whether population risk overall is compromised but rather whether the presence of these sensitive subgroups might unacceptably distort the distribution of that risk. For example, such unacceptable distortion would occur if 50% of risk overall were to be concentrated in a 'sensitive' population comprising 10% of the whole – in this situation the implementation of current standards in radiological protection would lead to under-protection of 10% of the population whilst perhaps over-protecting the remainder.

The data and computational modelling provided by the ICRP Task Group showed that familial cancer disorders were extremely rare in typical human populations (<1% of live births) such that it was biologically most improbable that they could be sufficiently sensitive to create an unacceptable distortion of population risks. It was however recognised that in a few inbred human populations the prevalence of such disorders might, in principle, be sufficiently high to create problems.

Individual risks: The Task Group provided data and arguments supporting the concept of increased sensitivity to radiation carcinogenesis in most familial cancer-predisposing disorders. Great uncertainty and variability of the degree of increased sensitivity was however recognised and stressed. For the purposes of illustrative calculation it was assumed that a hypothetical breast cancer gene conferred to the female carrier a risk that was 10 fold greater than normal.

For relative risk estimates the Task Group employed ICRP 60 estimates of normal female breast cancer risk, the 10 fold increase in risk for the hypothetical genetic case and current estimates of spontaneous life-time breast cancer incidence in such genetic cases. These calculations showed that low-dose irradiation (a life-time whole body dose of 100 mSv) would produce an undetectable excess risk in the hypothetical case principally

because the spontaneous life time risk was so high. Subsequent publications[4,5] have considered this low dose issue in the context of conventional medical diagnostic exposures. The principal conclusion is that the additional increment of risk to genetic cases will be substantially outweighed by the potential diagnostic benefit. The higher doses resulting from interventional radiology might require further consideration but, in all cases, informed clinical judgements should underpin decisions.

A similar approach was adopted by the Task Group to illustrate the effect of a 10 fold increase in sensitivity on absolute risk after 100 mSv, i.e. The excess cancer risk in the sensitive genetic case against that in a normal woman without any correction for the large differences in spontaneous breast cancer rates.

These illustrative calculations showed that although the absolute excess of breast cancer in the genetic case was increased 10 fold, when all organ sites were considered the overall increase in absolute cancer risk was only modest (~2 fold). This arises because, like many such disorders, heritable breast cancer does not have major implications for risk in organs other than the breast and sometimes the ovaries. This effect will tend to dilute the genetic impact on cancer risk overall but the Task Group were careful to point out that some known genetic disorders do show increased risk in multiple organs. Accordingly, this interim judgement needs to be reviewed periodically in the light of further data on the spectrum of excess tumours in the relevant genetic disorders.

In the same context the Task Group finally considered risk at high doses (2 Gy) and showed that, in principle, the relative risks can become significant. They identified post-radiotherapy second cancers as the principal source of concern and there are reasons to believe that high dose medical irradiation at young ages may pose the greatest risk to these genetic cases.

7. GENETIC TESTING IN THE CONTEXT OF RADIOLOGICAL PROTECTION

Through intense molecular studies many of the genes that determine human familial cancer have been isolated and characterised including DNA sequencing. The acquisition of these data has allowed the provision of so called genetic tests for the presence of specific germ line mutations that increase cancer risk. In a medical genetic context, these have proved most valuable in detecting the presence of the same inherited mutation amongst family members. There are however important ethical issues surrounding the use of such tests even in a medical genetics setting.

In radiological protection, the context of possible genetic testing will be different. First, in the vast majority of instances, those who might be considered for testing will be unrelated. Under these conditions and in order to obtain meaningful data, the number of different genes and different mutational sites that would have to be tested will be huge. Conventional methods do not have the capacity to deal with this problem. New and very rapid ‘DNA chip’ methods are now becoming available for these tests but the predictive value and economic viability of the whole scenario is open to question, particularly given the rarity of the known familial disorders and the small relative risk that applies for low dose exposures.

Overall, ICRP judged that it would be some time before such genetic testing might find a place in radiological protection. They doubted the future benefits that might accrue for genetic testing for cancer susceptibility in the context of occupational exposures but were

able to envisage some advantages prior to certain high dose medical exposures where there may be benefits for clinical management. Since however the whole issue of testing for genetic disorders has or will become subject to legislation in most developed countries, ICRP believe that their role in providing such judgements will be limited.

8. CONCLUDING REMARKS ON CURRENT AND FUTURE RESEARCH

It is becoming widely recognised that the acquisition of knowledge on genetically determined radiation risk will be of significant importance in the further development of radiological protection standards. At present, knowledge is insufficient to anticipate specific change but it is possible that this position may alter over the next decade. Of particular fundamental and practical importance are the low penetrance mutations which are likely to be relatively common but of which we know little. Conventional medical genetic and epidemiological approaches to these problems clearly lack the power of resolution that is necessary. For this reason, proof of principle experimental studies on the mechanisms underlying variation in DNA damage response and tumorigenic development offer the best immediate prospects and these were well represented in the EU nuclear fission research portfolio of the 4th Framework Programme. During the same period (1996-1999) other EU groups initiated work to seek evidence of low penetrance genes that may influence radiation tumorigenesis in mouse models of human neoplasia. In this area, the work whilst at an early stage of development, has succeeded in mapping naturally variant genes that can strongly influence post-irradiation tumour development; genetic loci associated with tumours of bone, skin, intestinal, breast and lympho-haemopoietic tissues received most attention. Even at this early stage, these studies are lending support to the concepts of a) the common occurrence of variant genes, b) the organ-specificity of low penetrance genes, and c) gene-gene and gene-environment interactions including in some instances cross sensitivity to different tumorigenic agents.

As noted above direct epidemiological and genetic approaches to cancer susceptibility after radiation remain problematical. Nevertheless further follow up of second tumours in RT patients with specific genetic disorders or known family histories of cancer would be valuable. As an alternative, it would be instructive to determine the extent to which adverse normal tissue reaction in RT patients from the general population might correlate with second tumour risk.

The whole areas of DNA damage response and genetic susceptibility to radiation were highlighted in the call for proposals in the 5th EU Framework Programme and further advances in conceptual understanding may be anticipated. Some of these concepts, when validated, are likely to contribute to the way in which radiological protection standards are developed in the future.

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Table 1. *Genetic disorders involving DNA processing defects*

Disorder	Genes/locus	Defect proposed	Major clinical features	Cancer	Approximate prevalence (per live birth)
Autosomal recessive					
Xeroderma pigmentosum	*XP-A to XP-G*	Excision or post-replication repair	Photosensitivity and cancer of UVR-exposed skin	Squamous cell carcinoma, basal cell carcinoma and melanoma	1 in 250 000
Cockayne syndrome	*CS-A, CS-B*	Transcribed strand repair	Photosensitivity, dwarfism	No excess	*
Trichothiodystrophy		Excision repair	Photosensitivity, abnormal, sulphur-deficient hair	No excess	*
Bloom syndrome	*BS*	DNA helicase?	Photosensitivity, dwarfism	Various	*
Ataxia-telangiectasia	*ATM*	kinase activity	Neurological defects, immunodeficiency	Lymphoma	1 in 100 000
Nijmegen breakage syndrome	*NBS*	p53 binding protein	Microencephaly, immunodeficiency	Lymphoma	*
Fanconi anaemia	*FA-A to FA-C*	DNA cross-link repair	Bone marrow deficiency, skeletal abnormalities	Leukaemia	1 in 300 000
Werner syndrome	*WS*	DNA helicase?	Accelerated ageing	Various	*
Autosomal dominant					
Hereditary non-polyposis colon cancer	*MLH1, MSH2, PMS1, PMS2*	DNA mismatch repair	Excess cancer	Colon cancer, endometrial cancer	1 in 2 000
Li-Fraumeni syndrome	*TP53* (others?)	DNA damage recognition	Excess cancer	Various	1 in 50 000
Ataxia-telangiectasia	*ATM*	Kinase activity	Excess cancer	Breast cancer?	1 in 200

*<1 in 100 000, some limited to a few individuals world-wide.

Table 2. *Principal familial cancer disorders involving tumour suppressor genes*

Disorder	Genes/locus	Defect proposed	Cancer	Approximate prevalence (per live birth)
Heritable breast/ovarian cancer	*BRCA-1* *BRCA-2* (others?)	Transcriptional regulation, DNA repair?	Breast/ovarian cancer Breast cancer (also male)	1 in 800 1 in 1 600
Familial prostate cancer	*HPC1**	?	Early onset prostate cancer	1 in 500
Familial adenomatous polyposis	*APC*	Transcriptional regulation	Colorectal cancer (multiple polyps)	1 in 8 000
von Hippel Lindau	*VHL*	Transcriptional regulation	Renal cancer	1 in 30 000
Wilms-Aniridia Denys Drash syndrome	*11p locus* *WT1*	? Transcriptional regulation	Nephroblastoma Nephroblastoma (+ others)	} 1 in 10 000
Neurofibromatosis type 1 Neurofibromatosis type 2	*NF-1* *NF-2*	GTPase regulation Cytoskeletal linkage	Neurofibroma Schwannoma Meningioma Neurofibroma	1 in 3 000 1 in 30 000
Nevoid basal cell carcinoma syndrome	*PTC*	Cell–cell interaction	Skin cancer Medulloblastoma	1 in 50 000
Familial melanoma	*MLM2*	?	Melanoma	?
Tuberous sclerosis	*TSC1* *TSC2*	?	Hartomas of skin, nervous tissue, heart and kidneys	1 in 20 000
Retinoblastoma	*RB1*	Transcriptional regulation	Retinal tumours, bone/soft tissue sarcoma, brain cancer and melanoma	1 in 25 000

*Accounting for a small proportion of heritable prostate cancer, no specific evidence for tumour suppressor function, much uncertainty on prevalence.

SCIENTIFIC SEMINAR ON GENETIC SUSCEPTIBILITY AND NEW EVOLUTIONS ON GENETIC RISK

Conclusions and potential implications

Dr J. PIECHOWSKI

PREAMBLE

This document presents the main conclusions and potential implications of the Scientific Seminar on Genetic Susceptibility and New Evolutions on Genetic Risk held in Luxembourg on 29 November 1999. While it is not intended to report, in an exhaustive manner, all the opinions that were expressed by the speakers or by the audience, it will take into account the discussions that found place during the subsequent meeting of the « Article 31 » Group of experts on 30 November 1999. The content of the document has been discussed within the RIHSS (Research Implications on Health Safety Standards) Working party[*] and has been submitted for advice to the lecturers, whose remarks were taken into account as far as possible, subject sometimes to the final arbitration of the RIHSS Working Party.

1. RIHSS SEMINARS : RATIONALE

The RIHSS Working Party of the « Article 31 » Group of experts was set up with the task to help to identify the potential implications of recent research results or new data analysis on the European Basic Safety Standards Directive (BSS), and on the related Recommendations and other guidance.

The adopted approach is the following : on the basis of the input from the Directorate General Research of the European Commission and of the information transmitted by the individual members of the « Article 31 » Group of experts, the Working Party proposes yearly to the « Article 31 » Group relevant themes that could be discussed during a subsequent seminar. After selection of a theme and approval of a draft programme by the « Article 31 » Group, the Working Party deals with the practical organisation. The seminars involve invited speakers, mainly leading experts, with the task of presenting a clear synthesis of the state-of-the-art in the field, with special attention to new information. Additional experts, indicated by the members of the « Article 31 » Group in their own country, take part in the seminars and act as peer reviewers. The seminars are convened by the Commission the day before a meeting of the « Article 31 » Group. Such organisation gives to the members of the « Article 31 » Group the opportunity to discuss the potential implications of consolidated scientific results.

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2. INTRODUCTION AND PURPOSE OF THE SEMINAR

Induction of cancer by ionizing radiation is a well-established fact. As the malignant properties of a cancerous cell are transmitted to its daughters, that means that the centre of the process is somewhere in the genetic material of the cell. Adaptive behaviour of the cancerous cells in tissues and the frequent evolution toward a higher level of malignancy emphasize the key-role of the genetic material. Some well-known hereditary diseases are associated with a high probability of occurrence of cancer and these cancer-prone genetic disorders have been shown to segregate in families of humans and experimental animals.

Typical modifications and abnormalities of genes leading to malignant transformation and, for some, their role in heritable cancer have been discovered by means of more and more sophisticated techniques of cell molecular biology and genetics. Similar discoveries have been made on the fundamental cause of non-cancer genetic disease.

Considering the potential influence of the characteristics of the genetic material on the predisposition of developing spontaneous and /or radiation-induced cancers and hereditary effects, updated information on the following topics is required :

- scientific knowledge on the fundamental biological mechanisms and, as far as possible, estimation of the risk of cancer and heritable disease ;
- methods for the detection of genetically enhanced radio-sensitivity and susceptibility to radiogenic cancer ;
- specific recommendation for protection and possible preventive actions.

3. BACKGROUND INFORMATION ON RADIATION AND CANCER

Radiation has miscellaneous initial targets in the tissues. The commitment of cells to malignancy still appears as a puzzling process and many factors may influence its evolution by promoting, delaying or even preventing it. They include the genetic and the health status of the individual as well as the influence of external factors such as environmental conditions, infections, food or particular life habits. The intrinsic properties of the genetic system of an individual are an essential component of the probabillity of commitment of cells to malignancy. The cancerous signature of the affected cells becomes a part of their genetic material. The intrinsic properties in question may be considered under two categories : those related to the induction of DNA molecular modification(s) leading to cancer ; and those related to the capability of either repairing DNA lesions or inducing programmed cell death (apoptosis) in case of deleterious modifications of the genetic material.

Some typical, well-identified genetic diseases are associated with a high risk of cancer occurring either apparently in a spontaneous way or as a result of the action of a chemical or physical agent. In the latter case there is generally a known or strongly suspected relationship between the type of disease and the triggering agent as for instance UV radiation with xeroderma pigmentosum (XP) and ionizing radiation with Li Fraumeni Syndrome (LFS) and with Ataxia Telangiectasia (AT).

Considering the general question of which genetic diseases may be associated with a higher risk of cancer, it is convenient to distinguish the following situations :

- defective expression of a dominant autosomal gene (in both sexes) ;
- defective expression of a X-linked gene (in males) ;
- defective expression of a pair of recessive genes -one inherited from the father and the other from the mother- (in both sexes) ;
- defective expression of multiple genes (in both sexes).

The first three situations correspond to simple genetic, usually called Mendelian diseases ; the fourth is related to the wide and complex group of multifactorial diseases.

In the first two situations, the occurrence of the disease is essentially deterministic and is predictable. In the third case, it is uncertain and less frequent because two genes must be simultaneously mutated. The situation is even more uncertain and complex for multifactorial diseases.

The genetic targets which trigger the malignant process have two characteristics : they do not damage the vital logistics of the cell but they frequently establish a permanent diversion in the management of its developmental life stages. A critical feature of the tumorigenic process is a disturbance in the balance between cell division and cellular differentiation. The ratio of cell divisions versus differentiation and normal maturation is highly increased. Accordingly, tumor development is frequently associated with mutations in genes that determine these cellular activities.

As to the radiation-sensitivity of individuals in a large population, it may be, as for most biological phenomena, that it follows a statistical distribution, say for convenience a roughly normal distribution. Coming back to the various genetic configurations causing either diseases or conditions predisposing to radio-genic cancers, i.e. expression of dominant, X-linked, recessive or multiple genes, it is clear that we move respectively from the right side (high probability) towards the left side (low probability) of the distribution. Individuals with typical Mendelian diseases like the radiosensitive disorder, ataxia-telangiectasia, are at the extreme right side of the distribution whereas the majority of apparently healthy individuals are in the region around the mean of the distribution.

4. TOPICS DEVELOPED DURING THE SEMINAR AND LESSONS LEARNED THEREFROM

Four presentations have been made which allow us to identify the following main features, being aware both of a possible partiality or oversimplified interpretation and of the rapid scientific and technical progress which may make some of them obsolete in a near future.

According to K. Sankaranarayanan, a considerable proportion of radiation-induced mutations is not compatible with life and will not be seen or expressed as diseases. Some other mutations are recoverable, but may not induce a disease or even a trivial phenotypic abnormality. Their expression may be weak or strong. These are some of the reasons why the observed risk of radiation-induced diseases is significantly lower than the expected risk based on appearance of radio-induced gene mutations.

The doubling dose (DD) is the dose which causes an increase in the natural mutation risk by a factor of two. The following relation applies :

DD x [induced mutation rate per Gy] = [natural mutation rate]

DD has been recently revised. In previous estimate, both factors of the above relation were based on mouse data, leading to DD = 1 Gy. The assessment has been improved using human instead of mice natural mutation rate and the revised DD is now 1.5 Gy. A recent proposal is to take into account a new factor expressing the human recoverability of the radiation-induced effect in livebirths. Although the present data are insufficient to assess with some robustness the numerical accuracy of the human recoverability factor, the situation may rapidly change with advances in the human genome project.

Simple genetic diseases are caused by mutations in single genes whereas multifactorial diseases depend on interaction between genetic and other factors such as environmental conditions, infections, food or particular life habits. The natural probability of simple genetic diseases is around 2.4 % ; that of multifactorial diseases is close to 65 % and that of congenital abnormalities is around 6 %. The new estimate of excess probability in the first generation progeny of an irradiated population lies between 0.05 and 0.1 % per Gy for the simple genetic diseases and between 0.02 and 0.08 % per Gy for the multifactorial diseases, and is of the order of 0.1 % per Gy for the congenital abnormalities.

Next to these endpoints, K. Sankaranarayanan has introduced a new notion, based on the fact that radiation-induced mutations are essentially multigene deletions occurring by random deposition of energy. This could result in the induction of congenital abnormalities, with an excess probability close to 0.1% per Gy, the natural probability being about 6%. Moreover, other currently not identified and as such not investigated phenotypes may also be induced.

The main features of chromosomal anomalies associated with malignant cell transformation, whether induced by radiation or not, were presented by L. Sabatier. Four cytogenetic types of cancer are identified each of them being associated with a particular set of malignancies :

- monosomic type in which anomalies may be deletions and chromosome losses leading essentially to epithelial cancers ;
- trisomic type characterized by a progressive increase of the number of chromosomes ; the associated cancers are essentially adenocarcinoma and non epithelial cancers ;
- translocation type, a balanced rearrangement which is exceptional in epithelial cancers but frequent in sarcomas ;
- pseudo-normal type, in some intestinal and breast cancers.

Ionizing radiation causes deletions, chromosome losses and chromosomal instability in the damaged cells and in their progeny. Heavy ions are clearly more deleterious than low LET radiation. The bystander effect is a particular phenomenon consisting of induced damages, including chromosomal instability, in non-irradiated cells located around the irradiated target cells. There is no well-established explanation for this observation.

Chromosomal instability is clearly associated with subsequent malignant cell transformation. However, the transformation does not appear immediately : many

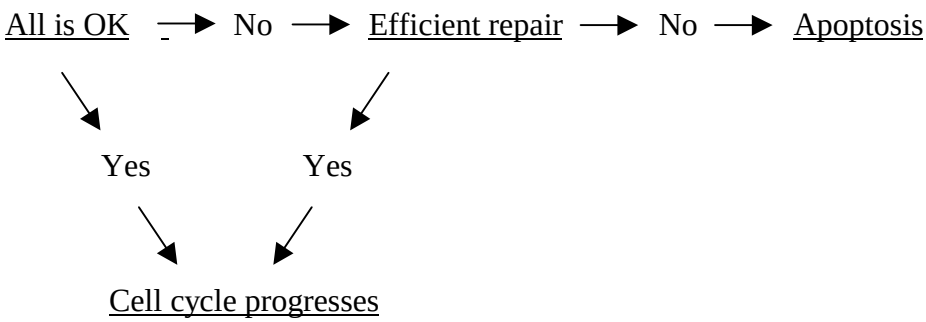
intermediate steps are needed. Because of these, a large time interval elapses between damage induction and the emergence of the cancerous state.

It is also well-established that anomalies of the behaviour and of the evolution of telomeres are involved in the chain of events leading to cancer. Telomeres are the extremities of chromosomes. They are elaborated through the enzymatic action of telomerases. In a normal cell lineage, telomeres get shorter after each cell division and cell division stops when telomeres have been totally pruned. Senescence is related to that phenomenon. In normal conditions, senescence leads to death in a non-reversible way. Conversely, in abnormal conditions like irradiation, some senescent cells undergo a genetic modification which allows them to remain in a latent state of survival during which telomerases reappear or become active again. Elaboration of new telomeres in a permanent way results in a non-reversible proliferation of the transformed cells, i.e. in continuous growth of clones that become populations of malignant cells. The underlying mechanism of that mitotic restoration remains to be explained.

Chromosomal instability associated with persistent presence of telomeres could play a critical role in induction of radiation-induced tumours.

Some important features of remedial mechanisms implemented by a cell to maintain its genetic integrity have been presented by J. Hoeijmakers. They consist of checkpoints located at strategic positions in the cell cycle and in enzymatic repair systems guided by specific proteins which detect the genetic lesions and repair them in an error-free or error-prone manner.

In normal healthy individuals, significant damage of the genetic material leads to activation of the checkpoints. The principle of control of integrity of the genetic material is illustrated in the following scheme. The basic functions of the checkpoints of a cell consist of carrying out a number of well-defined operations :



A high risk of cancer induction by radiation is observed in individuals suffering from inherited disorders associated with defects in cell cycle check point activation and in co-ordination of DNA repair, such as ataxia telangiectasia (AT) or Nijmegen breakage syndrome (NBS). However that does not mean that checkpoints are especially designed to detect genetic anomalies responsible of cancer. One can only say that certain anomalies, if not detected by checkpoints, lead to malignant transformation. Conversely, a number of anomalies predisposing to cancer are probably not detected by normal checkpoints. It is, with the current knowledge, difficult to have any confident estimate of

the effective role of the checkpoints in avoiding occurrence of cancer and in particular of radio-induced cancers.

The field of repair mechanisms is very large and complex. One of the more problematic lesion for a cell to repair is the double strand breakage of a chromosome (DSB). Ionizing radiation is very efficient to cause such lesions and oxygen enhances that noxious action. Various important chromosomal anomalies and aberrations result therefrom. Operational rescue of the hit chromosomes is carried out via two major DSB repair pathways :

- when cells have not replicated their DNA, DSB are repaired by a process called end-joining ; this mode of DSB repair joins DNA ends directly, without any intrinsic mechanism checking whether the correct ends are ligated, and consequently the probability of an erroneous joining is high ;

- a more favourable case corresponds to a situation where a cell has replicated its DNA. In this case, repair is achieved by a process designated homology-dependent recombination. This mechanism searches for the homologous intact sequences either on the sister chromatid or on the homologous chromosome. These sequences are used to align the broken ends and properly ligate them. The large overlap of the matched DNA sequences at the recombination site explains the rather low risk of error.

Both repair systems, end-joining or recombination repair of broken DNA strands, involve specific proteins which manage the operations. It has been recently shown that the proteins in question head towards the hit regions of the nucleus to locate the chromosomal fragments, to link the relevant DNA strands and to induce the repair process by activation of the enzymatic machinery. The genes coding for the synthesis of these proteins have been identified. In case of defect, the DSB repair process breaks down.

According to R. Cox, around 10 % of known Mendelian human genetic disorders show some association with cancer and for perhaps 100 such disorders the evidence is unambiguous.

No clear relationship can be established between risk of cell death after irradiation, generally called radiosensitivity and risk of spontaneous or radiation-induced cancer. That may be explained by the fact that cancer results from a very particular, *a priori* stochastic evolution of the hit genetic material ; the induction of such rare events in cells surviving radiation may not have a simple relationship with cell death. Post-irradiation genetic misrepair in cells may lead to direct death, to apoptosis or to survival with more or less abnormal genetic material. The latter situation is far from being exceptional. It is not *per se* the cause of cancer. Certain radiation-induced genetic anomalies result in disturbance of the DNA repair systems and of the cell cycle checkpoints. Such defects open the gate to the development of further genetic anomalies, these being related either to the ancestral irradiation or conversely to spontaneous *de novo* mutations. Some of these changes may lead, in a stochastic way, to non-reversible mutations determining the malignant phenotype. In this way cancer may be viewed as a multistep genetic process and the time interval between irradiation of a cell population and emergence of the cancerous clones may be very long.

A particular genetic situation is that concerning the pairs of specific recessive genes whose mutation can lead to cancer if both of them become defective. This is typically the case of retinoblastoma. Hereditary defect of one such gene reduces the target gene

number from two to one. Accordingly there is an increased probability of spontaneous and induced tumorigenic initiation usually without effects on cellular radiosensitivity as conventionally measured.

Less than 1 % of the population has strongly expressing genetic disorders associated with a very high probability of cancer, accounting for 5 to 10 % of all occurring cancers. Age of onset of malignancies in that subpopulation is strikingly low. Similar estimates concerning weakly expressing genes are not available and will probably not be available in a near future due to the lack of sufficient epidemiological data. Answer to that fundamental question would help in setting up a more comprehensive classification of the genetic conditions predisposing to cancer and their association with specific forms of malignant cell transformation.

The question about overall or organ specific susceptibility to cancer has to be considered carefully. Indeed it is necessary to distinguish on the one hand the well-identified diseases and on the other hand the weakly expressing and less obvious disorders associated with cancer predisposition. As to the former, it is clear that usually there is an organ or tissue specificity. That is explained by the specific involvement of one or more critical genes in the regulation of development and differentiation of the organ or tissue in question. Conversely, at present, nothing can *de facto* be said concerning the weakly expressing genotypes associated with cancer predisposition.

Estimating the risk for a given individual to be susceptible to radiation induced cancer is at present a unresolved question, except for individuals :

- with a clinically well-identified hereditary disease associated with a high cancer risk or,
- being member of a family strongly suspected of having such a disease or,
- possibly those experiencing an abnormal reaction after radiotherapy or interventional radiology.

Some new and very rapid « DNA-chip » methods are now becoming available for genetic testing but their predictive value and the economic aspect have to be improved before considering them for any current operational use. Finally, from a practical point of view, detecting sensitive individuals is still based on rather empiric approach than on reliable genetic tests. The very low relative risk for such individuals at low doses, typically at doses in the domain around the current limits, emphasizes the restricted practical use of the concept of genetic susceptibility to cancer. Indeed, that concept appears to be essential in particular circumstances concerning exposures above some tens of mGy, being aware of the fact that we are able to detect *a priori* only a limited number of sensitive individuals.

5. CONCLUSION

These conclusions will try to emphasize the practical consequences and potential implications of the updated but still limited knowledge on genetic susceptibility to radiation induced cancer. They may be split broadly into four parts :

1. in the domain of doses around the current limits either for public or for workers, there is at present no reason to modify the present principles and management of radiation protection ;

2. large uncertainties remain as regards the collective impact of low penetrance mutations, i.e. the weakly expressing ones, and of possible genotypes associated with overall rather than with specific organ cancer susceptibility. Some questions also remain as to the increase in individual risk at moderate doses and as to the age of onset of cancer for the cancer-prone individuals carrying relevant strongly expressing genes.

3. in occupational medicine, there is no actual routinely applicable method to identify workers who could be especially susceptible to radiation induced cancer, except for the rare and obvious diseases which are immediately detected by clinical examination or family history of cancer. It is the responsibility of the occupational physician to exclude such persons from any work involving significant exposure to ionizing radiation. This also concerns the members of emergency intervention teams. The possible future availability of screening tests for some genes related to cancer susceptibility will raise legal and ethical questions concerning their use.

4. the main conclusion is certainly related to protection of patients undergoing high level radiation exposures during radiotherapy, interventional radiology or some high-dose radio-diagnostic procedures. It would be very useful to elaborate a short guide for the general practitioners and for the specialists in surgery, cardiology, radiology and radiotherapy whose practices may potentially lead to high risk of exposure to radiation. Information should be given on the following topics :

   - identification of specific clinical symptoms,
   - questioning on eventual familial disorders,
   - when available, cytogenetic and / or cell bio-molecular examination in case of suspicion of some hereditary problem,
   - adapting the diagnostic or therapeutic procedure as appropriate,
   - when experience has been gained on the above topics, it could be possible to establish an efficient system of prevention.

ABSTRACT

It is estimated that about 5% of all cancers are related to predisposing germline mutations. There is evidence that certain germline mutations may also make the carrier more sensitive to ionising radiation and subsequent carcinogenesis. However, it is reasonable to assume that susceptibility to radiation-induced carcinogenesis behaves as a continuously varying feature due to segregation of multiple predisposing genes. Cancer and genetic research to better understand this issue are ongoing.

The presentations made at the seminar reviewed the existing knowledge on the effects of the characteristics of genetic material on the predisposition of developing radiation-induced diseases.

The subject was treated from the four points of view:

- Emerging perspectives in radiation genetic risk estimation
- DNA repair deficiency and its implications for human health
- Radiation-induced chromosomal instability
- Genetic susceptibility to cancer and its implications for radiological protection and research needs.

The publication is completed by considerations on the conclusions that can be drawn from the seminar and on the potential implications of the informations presented on the development of the European Union radiation protection legislation.

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