Genomic Instability Induced by Ionizing Radiation

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INTRODUCTION

In contrast to general assumptions it has frequently been shown that DNA per se is not a very stable molecule. Besides spontaneous changes due to the chemical structure of the macromolecule a number of endogenous as well as exogenous agents induce many changes of the DNA in living cells. Many lesions of the intracellular DNA can be induced by reactive oxygen species. These include DNA strand breaks and many different types of DNA base damage (1).

The stability of the genetic information of cells and organisms during individual life and over the generations is guarranteed mainly through very efficient DNA repair processes and other stabilizing mechanisms. However, these control mechanisms are not perfect. Therefore some damage persists. The consequence is that cells die or become mutated. The unrepaired damage can be expressed cf through chromosome breaks and other types of chromosomal aberrations. This phenomenon already occurs very early in the development of a mammalian organism. Thus chromosome aberrations have been observed in 2.3 % of the metaphases during division from the zygote (1-cell) to the 2-cell embryo, this number was 4.2 % for the division from the 2-cell to the 4-cell embryo and 7.7 % for the division from the 4-cell to the 8-cell embryo (2). Some of these breaks are certainly caused by the experimental treatment of the cells in order to prepare metaphases for the determination of chromosomal aberrations but the steady increase of the number of chromosomal lesions clearly show the participation of the DNA lesions which have been formed by intracellular processes.

It has been observed for many decades that ionizing radiation causes chromosomal damage and these lesions are suggested to be the primary events for a number of radiation effects which determine the radiation risk of cell death, mutagenesis and carcinogenesis. There is good experimental evidence for such a proposition. Especially carcinogenesis but also mutagenesis are multistep processes during which several mutations occur in a sequence in order to develop radiation late effects. Uptonow it was thought that the exposure to ionizing radiation initiates this chain of events by setting DNA lesions which then develop through cell proliferation and further independent mutations to the manifestation of cancer. For this understanding chromosomal damage has been studied mainly at or directly after the first cell division post radiation. During recent years it has been observed that chromosomal damage is expressed not only at the first cell division post radiation but also at the following cell divisions. It was furthermore found that after a first wave of chromosomal aberrations during the first cell divisions post radiation a later wave of chromosomal aberrations and other DNA lesions occur which are apparently independent from the first events. These later aberrations apparently originate from some general damage which affects the whole cellular genome. This phenomenon is called genomic instability (3,4,5).

CHROMOSOMAL ABERRATIONS AS DIRECT RADIATION DAMAGE

The induction of chromosomal aberrations by ionizing radiation have been extensively studied in many cell systems. With human cells investigations with lymphocytes are predominant (6,7). It has been shown that chromosome breaks and other types of aberrations can be observed very rapidly after exposure to ionizing radiations. The effects are dependent on radiation quality and dose rate. Good dose relationships are observed down to comparatively small radiation doses (in the range of 100 mSv or even less) (8). Therefore chromosomal aberrations and especially the determination of dicentric chromosomes in human lymphocytes have been investigated as an indicator system for radiation exposures after radiation accidents and dose estimates have been performed from such studies (9,10). As unstable chromosomal aberrations like dicentric chromosomes can get lost from the cell nucleus during mitotic cell division, it is convenient or even necessary to determine the aberrations during the first mitosis after irradiation.

However, it has been observed that further and new chromosomal aberrations are formed during the following cell cycles and are expressed at the subsequent mitotic cell divisions. This effect has been intensively studied with preimplantation mouse embryos (2). In these embryos the number of cell divisions can be observed in a very definite way. When the zygote (1-cell stage) is irradiated, one knows that the cells of the embryo in the 2-cell stage have divided once, in the 4-cell stage they have divided twice etc. Thus the occurrence of chromosome aberrations has been measured in the first three mitotic cell divisions after exposure to X-rays as well as neutrons. With both radiation qualities dominantly chromatid breaks are observed at the later mitotic cell divisions, whereas chromosome breaks are seen in the first post radiation mitotic division (11).

CHROMOSOMAL ABERRATIONS AT LATER STAGES AFTER IRRADIATION

In further experiments mated mice were X-irradiated 1 hour after the conception had taken place. The zygotes developped to complete fetuses which were obtained shortly before birth. Skin biopsies were taken and brought into cell culture. Without any further irradiation metaphases were prepared from the growing fibroblasts 48 hours later and chromosomal aberrations were measured (12). More chromosomal aberrations were observed in the fibroblasts which originated from fetuses irradiated in the zygote stage than in the fibroblasts from fetuses without irradiation. This effect was even higher in fibroblasts from fetuses which had developped a specific malformation (gastrochisis) after irradiation (Table 1).

Table 1
Percentage of metaphases with chromosome aberrations in fetal mouse fibroblasts after 48 hours cell culture. Cont: without X-irradiation; Norm: X-irradiation of the zygotes with 2 Gy, normal development; Gast: X-irradiation of the zygotes with 2 Gy, formation of a gastroschisis (11).

Fetus type	No. of studied Metaphases	Percentage of metaph. with aberr.	P-value
Cont	400	5.5	
Norm	322	12.1	< 0.01
Gast	503	17.3	< 0.01

These chromosomal aberrations could not have been developped directly from radiation lesions in the DNA. With such DNA damage in the zygote it would not have been possible that a whole fetus developped in utero. Therefore a different mechanism must have been responsible for this effect. Apparently over more than 30 cell generations some radiation-induced lesion must have developped which leads to a genome instability so that an increase of chromosomal aberrations occurs during the fibroblast incubation.

Since these surprising observations a number of further reports have been published where a radiation-induced genomic instability has been observed mainly after irradiation of cells in vitro (4,5,12). Not only delayed chromosomal aberrations in the progeny of cells surviving acute exposures to ionizing radiation have been described but also delayed reproductive cell death, reduction in plating efficiency, delayed mutations, transformations and other effects.

Little et al. (13) have studied the induction of delayed mutations in the HPRT-gene of CHO-cells. The cells were irradiated with 100 kV X-rays or α-particles from a plutonium-238 source. After irradiation the cells were plated and after about 23 cell generations macroscopic clones were isolated and the cells were reseeded for each clone in separate dishes. After a further incubation time in order to allow cell proliferation the mutation frequencies in the HPRT-gene were determined. In Table 2 the number of clones with a mutation frequency $<0.3 \times 10^{-6}$ and $10 – 1{,}000 \times 10^{-6}$ are presented.

Table 2
Frequency of mutations in clonal populations from single cells surviving irradiation (13)

Treatment	Total number of clones examined	Number of clones (percentage of total) $< 0.3 \times 10^{-6}$	$10\text{-}1{,}000 \times 10^{-6}$
Control (0 Gy)	256	170 (66 %)	2 (0.8 %)
X-rays (4 – 12 Gy)	300	142 (47 %)	25 (8.3 %)
α-particle	146	66 (45 %)	13 (8.9 %)

The data show that within the group of delayed mutations almost 10 % of the surviving cells have mutation frequencies which are up to 10,000 times higher than in the unirradiated cells. Further Little et al. (13) observed that the delayed mutations with the high frequencies have small deletions while for the mutations which appear directly after irradiation large deletions are observed. Thus, also the molecular spectrum, the

quality of the mutations differ. These data show that after irradiation the direct mutations and the delayed mutations clearly develop through different mechanisms. The delayed mutations have the characteristics of the spontaneous mutations. This is also the case for the delayed chromosomal aberrations (3). The dependence of induction of genomic instability on radiation dose is not clear uptonow. Taken all experiments together it seems that an increase of the frequency and degree of genomic instability exists in a dose range below 2 Gy of low LET radiation. After higher doses a plateau is reached.

PERSISTENCE OF GENOMIC INSTABILITY

Studies with genomic instability with cells in vitro have demonstrated that the radiation-induced genomic instability persist over many cell generations, a decrease has not been observed. In a mouse strain the development of a genetically predisposed malformation (gastroschisis) and the induction of genomic instability measured with chromosomal aberrations have been studied concomittantly (3,14). It was found that mice which develop the malformation after irradiation during the zygote stage also develop other skeletal malformations which are usually very rare with a significantly higher frequency. Further the frequency of gastroschisis and other developmental defects are significantly increased in the next mouse generation although no further radiation exposure took place (15). This means that this type of genomic instability is inherited to the next mouse generation although the transmitted effect is smaller than in the first generation. Interestingly mother mice who carry a malformed fetus carry more frequently two malformed fetuses in the second generation than in the first generation (Table 3).

Table 3
Distribution of malformations among mated females of the first and second generation (15)

Mated Females of	Females with at least one malformation	Females with 1 malform.	Females with 2 malform.	Females with 3 malform.
1st generation				
Control	**18**	**17**	**1**	
Exposed	**46**	**42**	**3**	**1**
2nd generation				
Control	**7**	**6**	**1**	
Exposed	**21**	**12**	**9***	

***Significantly different at P<0.05 when compared to the controls and at P<0.01 when compared to the first generation.**

Another example of development of chromosome instability in vivo has been demonstrated by experiments in which irradiated male bone marrow cells were transplanted into conditioned female mice in order to reconstitute their blood forming system in the bone marrow after irradiation (12). An increased genomic instability was observed in 10 % of the bone marrow cells of donor origin up to one year after transplantation.

Several syndromes with a genetic predisposition for increased radiosensitivity show an enhanced chromosomal fragility (16). Thus the rate of micronulei can be increased. Micronuclei originate from whole chromosomes or from acentric fragments. It is possible to distinguish between these mechanisms by labelling the centromeres with corresponding DNA probes. The analysis with these methods shows that micronuclei in human lymphocytes carry between 70 to 80 % whole chromosomes. After irradiation the number of acentric chromosomal fragments increases and therefore less micronuclei carry a signal for a centromer. The same is the case when the instability of the chromosomes increases. A study of lymphocytes from uranium miners who had received high exposures to radon and daughter products as well as to dust with uranium in the forties and fifties resulted in a relative decrease of micronuclei with centromers. This could not be caused by the ionizing radiation directly as the time period between the exposure and the analysis was too large but the effect must be interpreted

by an increased genomic instability. This was underlined by the determination of chromosome aberrations. These effects were even larger when lymphocytes of uranium miners with a bronchial carcinoma were investigated (Table 4) (17). It is quite reasonable for these cases that after the radiation exposure a genomic instability had developped which promoted the formation of the malignancy.

Table 4
Number of micronuclei per binucleated cell (MN/BNC) (A) and MN with centromers (B) in lymphocytes of 5 healthy persons (H.P.), 14 healthy uranium miners (U.M.) and 14 uranium miners with bronchial cancer (U.M.C.) (17)

		H.P.	U.M.	U.M.C.
(A)				
MN/BNC	**Average value**	**18.8**	**21.4**	**42.4**
	S.D.	**3.9**	**10.2**	**39.3**
	P (t-test)	**-**	**n.s.**	**n.s.**
(B)				
MN with centromers	**Average value**	**76.4**	**62.1**	**54.9**
	S.D.	**3.1**	**3.5**	**3.1**
	P (t-test)	**-**	**<0.0001**	**<0.0001**

MECHANISM OF INDUCTION OF GENOMIC INSTABILITY

At present the mechanism of induction of genomic instability by ionizing radiation is not understood. It is also not clear whether only one mechanism exists for the various endpoints. Genomic instability is apparently not directly connected with a well-defined gene or chromosomes, it appears as a general phenomenon in which the whole genome is affected. Many possibilities are discussed. As the frequency of this effect is higher than the rate of mutations on the cellular level, an epigenetic mechanism has been discussed (4,12). Also the involvement of free radicals especially reactive oxygen species has been proposed. Further there is an indication that cells can develop genomic instability after exposure of the cell culture to α-particles without a direct traversal of that individual cell by a particle (18) . This type of "bystander" effect has further to be elucidated. Further attractive proposals are that the development of genomic instability is connected to the structure of telomers which stabilize the structure of chromosomes or to imprinting processes in the DNA which are involved in regulating the activation and deactivation of genes (19). DNA repair may be impaired under these conditions. In this connection it is of interest that quite a number of syndromes with increased radiosensitivity through genetic predisposition also show an increased genomic instability.

BIOLOGICAL IMPLICATIONS OF GENOMIC INSTABILITY

All stochastic late effects of ionizing radiation are multistep processes. Best defined are these events for carcinogenesis. Many experimental and clinical studies have demonstrated that cancer develops through several sequential mutation steps. Such a model has been proposed for colorectal cancer (20). For such a model the development of genomic instability would be of a very high significance, as these phenomena increase the mutation frequency and therefore increase the probability of a second or third mutation in an individual cell. As mutation frequencies are usually very low even after irradiation, genomic instability would facilitate the multistep processes considerably when the mutation frequency can be increased by a factor of 10 to 10,000 as it has been shown. The increased genomic instability in individuals with a genetically predisposed higher radiosensitivity may also explain partly the higher cancer risk in these individuals (16).

Therefore the evaluation of the mechanisms how genomic instability develops after irradiatian would be extremely important in order to improve our understanding of the mechanisms of carcinogenesis especially and stochastic radiation late effects in general. This is essential for a better evaluation of radiation risks in the low dose range. Finally it should be stressed that a better understanding of these processes will not increase risk factors as the evaluation of mechanisms and their knowledge does not enhance the rate of the late effects.

SUMMARY AND CONCLUSIONS

1. The induction of genome instability by low as well as high LET radiation has been demonstrated for various endpoints (e.g. chromosomal aberrations, mutations, delayed cell death) with cells in vitro and in vivo systems.
2. The genomic instability is not limited to a specific chromosome or gene. It can be transmitted over many cell generations and it can be inherited to the next generation in mammals. An induction in germ cells is possible.
3. A dose response has been found for the dose range of 0.5 to 2.0 Gy of low LET radiation. Data in the lower dose region are missing. They have to be obtained by extrapolation and have the usual uncertainties. In the higher dose range apparently a plateau of genomic instability is reached.
4. The mechanisms of the induction of genomic instability are not understood until now. A number of possibilities have been proposed including epigenetic, metabolic processes, reduced repai, changes of imprinting as well as telomers.
5. Genomic instability and the understanding of the mechanisms will help to gain a better knowledge of the processes how stochastic radiation effects with multistep mutation processes especially carcinogenesis develop and to improve the evaluation of radiation risk in the low dose range.

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