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Radiation-specific DNA non-double strand break lesions: repair mechanisms and biological effects

(Non-DSB Lesions)

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Final report
(summary)

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Project coordinator

L. H. F. Mullenders, Leiden University Medical Centre (LUMC), Department of Toxicogenetics, NL

Project partners

P Jeggo, A.Carr, and A. lehman, Genome Damage and Stability Centre, University of Sussex, UK

J. Hoeijmakers, R. Kanaar, and D. van Gent, Department of Cell Biology and Genetics, Erasmus University, NL

G. Almouzni and D. Papadopoulo, Institut Curie, FR

J. Thacker and P. O'Neill, MRC, Radiation and Genome Stability Unit, UK

F. Eckardt-Schupp, GSF, DE

S. Boiteux, CEA, FR

Objectives

To make rational judgements in radiation protection, it is necessary to extrapolate from the biological effects of radiation at low doses and low dose rates, and to have an appreciation of variation in response to ionising radiation (IR) among the human population. Therefore, a detailed knowledge of the basic mechanisms by which radiation induces cancer and genetic disorders is essential. DNA damage induced by ionising radiations is formed by direct energy deposition in DNA and by water radicals generated in the vicinity of DNA. The nature of ionising radiation-induced DNA lesions (such as 8-oxo-guanine, thymine glycols and single strand DNA breaks (SSBs)) overlaps substantially with lesions produced by endogenous oxidative metabolism in unirradiated cells. These endogenous damages are effectively repaired by the base excision repair (BER) pathway and this has led to suggest a threshold effect for radiation risk at low dose exposure. However, there is now clear evidence that the random energy deposition by IR not only induces isolated single DNA lesions but in addition a unique form of DNA lesions termed clustered DNA damage. This type of damage consists of two or more closely spaced lesions formed within about one helical turn in the DNA backbone and may include different combinations of base lesions and single strand breaks. The existence of clustered DNA damage caused by ionising radiation was first predicted from theoretical studies of radiation track structures and later demonstrated experimentally. The formation of clustered damage distinguishes ionising radiation-induced damage from normal endogenous damage. If processing of clustered DNA base damage differs from endogenous damage, then the linear-no threshold model might be the most appropriate model for the risk assessment of adverse effects of ionising radiation. Hence it is important to unravel the mechanisms underlying the biological effects of clustered DNA base damage-induced radiation exposures and allow better quantification of the risks of radiation.

Strategic aspects and research performed

In order to achieve the goals of the current project, we utilised the following general approach:

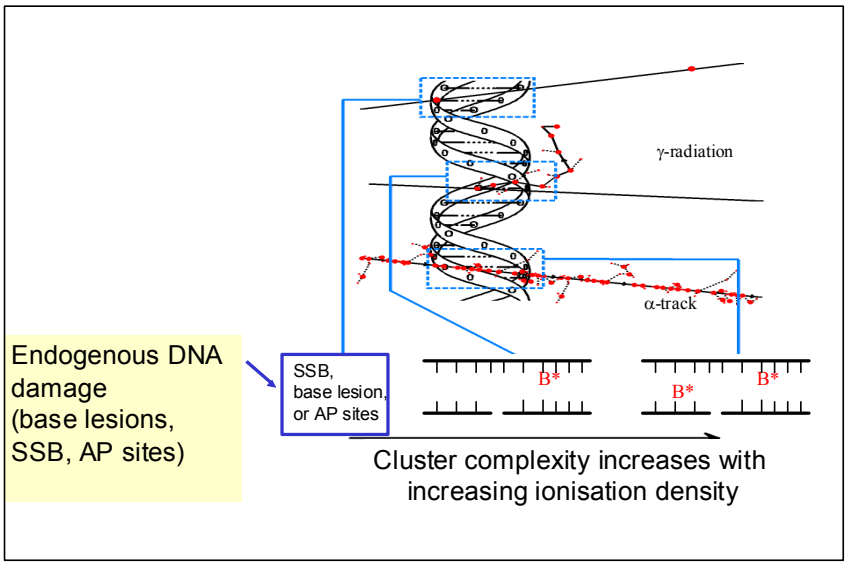
(1) different treatments to induced non-DSB damage (IR, UVA, hydrogen peroxide)
(2) different organisms/cell lines (human, mouse, chicken, yeast) with defined mutations in relevant repair pathways and cell-cycle checkpoints to dissect the mechanistic pathways
(3) genetics and biochemistry to investigate the precise functions of the proteins involved in the various repair pathways and their response to DNA damage using novel techniques (fluorescent redistribution after photo-bleaching, chromatin assemblage at individual DNA molecules, local damage induction)
(4) measurement of the fidelity of repair and the role of translesion synthesis employing cells and transgenic mice with defined mutations in repair genes and substrates with defined lesions
(5) high-density microarrays to obtain a catalogue of genes that respond to non-DSB damage
(6) ultimately, our goal was to understand the consequences of deficiencies in radiation damage responses in man. To this end, we aimed to use our knowledge to screen for polymorphisms in damage response genes within the human population and expression/mutagenesis in human tumour material.

The research carried out in this project was organised in the following five work packages:

- Work package 1: Nature and repair of radiation-induced DNA non-double strand break lesions;
- Work package 2: Interactions of radiation-induced DNA non-double strand break lesions with replication and transcription;
- Work package 3: Role of chromatin structure in repair of radiation-induced DNA non-double strand break lesions;
- Work package 4: Biological effects of radiation-induced DNA non-double strand break lesions;
- Work package 5: Stress responses dependent on DNA non-double strand break lesions.

Main achievements

IR-induced DNA damage constitutes a broad spectrum of purine and pyrimidine modifications, sites of base loss, single strand DNA breaks (SSB) of different kinds (see figure below). We have studied the repair mechanism and biological consequences of these modifications either as single entities or as clusters of DNA damage.



From single lesions to clustered damage

Defect in SSB repair may lead to a neurodegenerative disease. This project has contributed with much new information concerning how one type of lesions, i.e. chromosomal SSBs, is repaired in mammalian cells. We have expressed and purified two new human repair proteins (TDP1 and aprataxin). In addition, this project has contributed to the identification of a defect in SSB repair in a neurodegenerative disease, raising the possibility that SSBs are a significant factor in the etiology of neurological disease, possibly including non-pathological conditions such as ageing.

Frequency of IR-induced DNA base damage in human cells is relatively low. We have demonstrated by different approaches that the frequency of IR-induced DNA base damage in human cells is relatively low and certainly not high enough to explain some of the hazardous effects of IR by known mechanisms. Although low in number, the clustering of IR-induced

DNA base damage might lead to serious problems to cells and organisms as we have shown that clustered DNA lesions pose a significant challenge to repair systems and replication.

Clustered damage sites might pose problems to repair. We studied the reparability of clustered lesions by different BER pathways and showed that the damaging effect of such lesions depends on the sequence in which they occur as well as on the relative positioning of primary lesions within a cluster. The processing of clustered DNA damage (using cell extracts) is significantly retarded when compared with the rate of processing of the individual lesions. Some lesions such as 8-oxoguanine within a repair gap do not inhibit short-patch BER, however they block long-patch repair. In contrast, thymine glycol is a much more harmful lesion: when located within a cluster, thymine glycol causes a substantial delay in short-patch BER of the opposing lesion. We also find that repair of tandem lesions (2 or more DNA lesions at short distance on the same DNA strand) could lead to accumulation of strand breaks during repair. Generally the processing of the DNA lesions occurs sequentially thereby minimising the formation of potentially lethal DNA double strand breaks (DSB). This was confirmed in mammalian cells where it was estimated that approximately 15 % of the clustered damage sites are converted into DSB. We also found that DSBs can arise after treatment with agents that generate ROS. Such DSBs can have "dirty" ends that require additional processing prior to rejoining and specific genetic factors for repair.

Clustered damage sites might be highly mutagenic and thereby contribute to the health consequences of low-dose radiation. The type of clusters which are converted into DSB, contains 2 or more DNA lesions on opposite strands of the DNA double helix (bistranded clusters) and may cause infidelity of repair, resulting in mutations, as well as deletions and chromosome rearrangements. These genetic changes are known to play key roles in the multiple steps of the process leading to cancer. That delays in processing of repair intermediates can cause a significant increase in genomic instability and can affect cellular resistance to IR was evident from the observation that clustered damage sites are highly mutagenic relative to the individual lesions and that 2 AP-containing bistranded clusters are precursors to formation of DSB. These mutation studies confirmed that clustered damage sites might be highly mutagenic and thereby contribute to the health consequences of low-dose radiation. These findings are relevant to developing mechanistic models of low dose effects. At low doses of radiation (due to the higher yields of radiation-induced clustered damage to that of DSB) the probability that only a non-DSB clustered site or a single DSB is induced in any one cell is high relative to higher doses when a mixture of both types of damage are formed in any given cell. Overall, our data suggest that clustered lesions are repaired slower than single lesions of the same type and are likely to be responsible for the deleterious effect of IR. During the course of the project, it became clear from studies with mammalian cells lacking certain BER proteins, that non-repaired DNA base damage i.e. IR-induced oxidised pyrimidines, confers severe radiosensitivity to cells (An et al., 2005).

Radiosensitivity is most likely not related to transcription blocks. Impaired transcription of genes, of which expression is essential for viability, by IR-induced base damage has been suggested to be a serious threat to the cell leading to radiosensitivity and aging. Cells have developed a specialised repair pathway that preferentially removes transcription-blocking lesions called transcription-coupled repair (TCR). Although TCR deficiency might leads to radiosensitivity, both experiments with TCR deficient cells and the known IR-induced lesion frequency make it unlikely that interference of IR induced base damage with transcription plays a major role in the toxic effects of IR. The transcription response to base damage appears to act mainly through the stress-activated MAP kinase pathways and be caused by

oxidative damage. The characterisation of genes induced in this way was initiated. Surprisingly, the most dramatically induced gene did not lead to a sensitivity phenotype, whereas the less induced gene did. Ultimately we anticipate that new players in the response to IR-induced oxidative stress will be identified and used to characterise the non-DSB response to IR.

Transcription response: indications that oxidative damage plays a role. The transcription response to base damage appears to act mainly through the stress activated MAP kinase pathways and be caused by oxidative damage. The characterisation of genes induced in this way was initiated. Surprisingly, the most dramatically induced gene did not lead to a sensitivity phenotype, whereas the less induced gene did. Ultimately we anticipate that new players in the response to ionising radiation-induced oxidative stress will be identified and used to characterise the non-DSB response to ionising radiation.

We assessed the gene expression profiling of IR exposed human lymphocytes from 39 individuals to investigate the radiation response (2 Gy X-rays, 0.5 Gy/min). Overall fold changes were not dramatic. Only few genes (4.2 % or 940 genes per individual) had changed more than twofold after irradiation. The mean of the magnitude of changes is approximately 1.2-fold. It has been shown before that genotoxic stress induces substantial alterations at transcription level. The 200 most significantly changed genes had an average fold-change of 1.3. There is much variation in the radiation response of the individuals but the overall radiation response turned out to be largely comparable to other studies. All patients had a p53 dependent response and the apoptotic pathway seemed to be turned on, judging by the up- and down-regulated genes.

Polymorphisms in damage response genes within the human population and expression/mutagenesis in human tumour material. At the beginning of the project we intended to focus on cancer analysis for the BER gene Ogg1, since somatic mutations including several polymorphisms in hOgg1 have been identified in a fraction (< 5 %) of human lung and kidney tumour. The involvement of these alterations of hOgg1 in the cancer process is not yet clear. We suggested that inactivation of hOgg1 may be involved in late stages of carcinogenesis. Unfortunately, inactivation of Ogg1 in mice leads to the accumulation of 8-OxoG in the liver, but not to increased tumorigenesis. This disappointing finding has set the priority on other more promising mechanistic issues in the project.

Implications and future perspectives

The picture that emerges is that the ionising radiation induces clustered DNA damage that poses special problems to the cell both with respect to repair by BER and perhaps (during replication) by other repair systems such as recombination repair. Both the impaired repair and replication errors lead to mutagenic events and hence there is a requirement for further investigation into frequencies of clustered lesions by various radiation qualities and DNA repair mechanisms. To understand more precisely the hazardous impact of clustered DNA base damage for cellular function warrants model DNA templates harbouring defined clustered DNA base damage and various *in vivo* radiation protocols that enriched for clustered DNA base damage. This strategy will allow assessment of the biological consequences of limited (or even a single cluster, equivalent to the lowest dose possible) numbers of DNA base damage for the cell in terms of reparability, toxicity and mutagenesis. In addition, cell lines with knockdown of BER genes will provide the tools to study the role of these genes in

counteracting hazardous effects of IR exposure. Further development of mouse models with defects in these genes provide further the way to understand the role played by these genes in mitigating the development of cancer. Indeed, novel findings point to an important role of BER genes in radiosensitivity. In addition, screens for polymorphisms in these and other genes involved in radiation responses might reveal enhanced risk for cancer and ultimately identify individual susceptibility to IR.

Exploitation and dissemination

The results obtained in this period of the contract have been reported in 49 publications in peer-reviewed journals and presented at numerous national and international scientific meetings.