Review

Formation of clustered DNA Damage after High-LET Irradiation: A review

Megumi Hada[1,2] and Alexandros G. Georgakilas[3*]

[1]*NASA Johnson Space Center, Houston, Texas 77058, USA*

[2]*Universities space Research Association, Houston, Texas 77058, USA*

[3]*Department of Biology, Thomas Harriot College of Arts and Sciences, East Carolina University, Greenville NC 27858, USA*

[*]Corresponding author: Alexandros G. Georgakilas

Address: Biology Department, Howell Science Complex, East Carolina University, Greenville, NC.

Tel: 252-328-5446, Fax: 252-328-4178

e-mail: georgakilasa@ecu.edu

Formation of clustered DNA Damage after High-LET Irradiation: A review

Clustered DNA Damage/High-LET Radiations

Radiation can cause as well as cure cancer. The risk of developing radiation-induced cancer has traditionally been estimated from cancer incidence among survivors of the atomic bombs in Hiroshima and Nagasaki 1). These data provide the best estimate of human cancer risk over the dose range for low linear energy transfer (LET) radiations, such as X- or γ-rays. The situation of estimating the real biological effects becomes even more difficult in the case of high LET particles encountered in space or as the result of domestic exposure to α-particles from radon gas emitters or other radioactive emitters like uranium-238.

Complex DNA damage, i.e., the signature of high-LET radiations comprises by closely spaced DNA lesions forming a cluster of DNA damage. The two basic groups of complex DNA damage are double strand breaks (DSBs) and non-DSB oxidative clustered DNA lesions (OCDL). Theoretical analysis and experimental evidence suggest there is increased complexity and severity of complex DNA damage with increasing LET (linear energy transfer) and a high mutagenic or carcinogenic potential. Data available on the formation of clustered DNA damage (DSBs and OCDL) by high-LET radiations are often controversial suggesting a variable response to dose and type of radiation. The chemical nature and cellular repair mechanisms of complex DNA damage have been much less characterized than those of isolated DNA lesions like an oxidized base or a single strand break especially in the case of high-LET radiation. This review will focus on the induction of clustered DNA damage by high-LET radiations presenting the earlier and recent relative data.

INTRODUCTION TO THE IDEA OF CLUSTERED DNA DAMAGE

The 'idea' of clustered DNA damage was first introduced by Ward as *locally multiple damaged sites* (LMDS), i.e., several closely spaced damages within a short DNA segment that could be produced by ionizing radiation [2,3]. Ward introduced the idea of clustered DNA lesions to account for the increased lethality induced by ionizing radiation, which cannot be fully explained by the amount of double stand breaks formed, although the specific lesions, if unrepaired or misrepaired, can lead to a lethal event.

Random energy deposition by ionizing radiation induces a wide variety of DNA lesions [single (SSBs) and double strand breaks (DSBs), oxidized bases and apurinic-apyrimidinic (abasic, AP) sites] [4]. Ionizing radiation induces damage in DNA by direct ionization and through generation of hydroxyl radicals that attack DNA and induce some or all of the above lesions (indirectly). In addition to the prompt breaks induced by radiation, some post-irradiation ones can be also formed as the result of the attempted repair of some sugar and base residues induced directly which can later be converted to SSBs or DSBs [5]. Two or more DNA lesions of the same or different nature may be produced in close proximity to each other on opposite DNA strands (bistranded lesions), generally within one-two helical turns of the DNA molecule. Theoretical [6] and experimental studies [7] support the induction of these clustered DNA lesions in the cellular environment or high scavenger conditions by a single radiation track and not by multiple track events. These various closely spaced bistranded types of DNA damage are called clustered DNA damages and can include SSBs of varying complexity, oxidized base lesions (oxypurines and oxypyrimidines: oxybases) and regular as well as oxidized AP sites (Fig. 1). In general clustered DNA damage can be separated to two major groups: DSBs and non-DSB oxidative clustered DNA lesions, OCDL [8]. Even at doses as low as ~1 Gy (100 rad) ionizing radiation is capable of inducing all of the above types of DNA damage in the form of isolated lesions as well as in the form of clustered ones (1-10 bp apart) 9,10). Except ionizing radiation, other radiomimetic drugs like bleomycin or neocarzinostatin have been shown to induce DNA damage in a form of a cluster explaining in part the high toxicity of these drugs 11,12). It has been shown that clustered lesions constitute 50-80% of the total complex DNA damage 13). Simultaneous processing of the lesions located on opposite strands may generate additional DSBs in addition to the ones directly induced by ionizing radiation and enhance genomic instability 14,15). In fact, *E. coli* and rodent cells do generate *de novo* DSBs that could result from processing of bistranded clustered DNA lesions 1619). There are very limited data on the possible accumulation of OCDLs in human cells or tissues and in mammalian/human tissues exposed to ionizing radiation 20). Gollapalle *et al.* 8) showed for the first time that endogenous OCDL can be detected in non-irradiated mice tissues at a steady state of ~0.3-0.8 clusters/Mbp and that exposure of these tissues to ionizing radiation (X-rays) leads to an accumulation of DNA clusters detected 20 weeks after irradiation. Measurement of endogenous OCDL in the human breast cancer line MCF-7 revealed elevated levels compared with the non-malignant MCF-10A. These cluster frequencies are higher than the ones detected for DNA isolated from human skin primary cell cultures (20-40 clusters/Gbp or 0.02-0.04 clusters/Mbp) 21). These yields discrepancies may be due to different cell types or different isolation and enzyme-treatment methods for DNA analysis.

Cytotoxic effects of ionizing radiation are thought to result principally from incompletely or incorrectly repaired DNA lesions 22). While isolated damages are generally repaired efficiently, clustered DNA lesions have been suggested to be more difficult to repair, and in general are considered as DNA damages that are repair-resistant or non-repairable with a high mutagenic potential and, therefore, considered as highly significant biological endpoints 2,9). A significant number of studies suggest that the mutation frequency increases the closer the spacing of the clustered DNA lesions 2325). DNA clusters could be resistant to processing by glycosylases or endonucleases, as shown for synthetic oligonucleotides containing clusters of specific composition and configuration 2632). The majority of references on repair of clusters reflect retarded enzymatic activity. Such repair-resistant clusters could persist for a substantial time after irradiation 33,34).

PRINCIPLES OF MEASUREMENT OF CLUSTERED DNA LESIONS

Here we aim to give the principles of the approaches used from different laboratories for the detection of bistranded clustered DNA lesions as well as the general description of the way that they were evolved and fine-tuned for an efficient detection of closely spaced opposed oxidative DNA lesions in DNA isolated from human or mammalian cells, genomic DNA like T7 or λ-DNA or supercoiled DNA 35,36).

A unique approach for quantifying these types of bistranded damage in human cells using DNA repair enzymes isolated from *E. coli* was initially developed by Sutherland *et al.* 10). The idea relies on the fact that repair enzymes participating in base excision repair (BER) like DNA glycosylases and AP endonucleases will function also *in vitro*, i.e., on isolated DNA carrying clustered lesions. Once they detect the lesion (Fig. 1 and Table 1) in each cluster, they will excise it and cleave the DNA strand by their intrinsic lyase activity (DNA glycosylases) or cleave directly in the case of an AP endonuclease and create a SSB in each strand, i.e., a DSB in the case of a cluster (Fig. 1). As shown in Table 1, there is an overlapping specificity of Fpg, EndoIII and EndoIV towards abasic sites as well as between Fpg and EndoIII towards some types of oxidized bases. The additional DSBs induced by the repair enzymes can be measured using constant field or pulsed field gel electrophoresis (PFGE) following number average length analysis (NALA) according to the size of extracted DNA and are considered equal to the clusters detected by the enzyme [35,37]. Gels are stained with ethidium bromide, destained appropriately, and an electronic image is obtained using usually a CCD camera. Electronic images can be then processed using a densitometric software and a densitogram is obtained for each gel lane. A DNA dispersion curve relating DNA length to electrophoretic mobility, based on all length standards is determined from an analytical mobility function. From the profiles of irradiated and unirradiated (or enzyme and untreated DNA populations), the number average length, $\overline{L}$, of each DNA distribution is calculated and the DSBs or clusters will be calculated as described in (Fig. 1) [35].

In addition to the above approach using repair enzymes as damage probes, Georgakilas *et al.* [30,33] have used polyamines (putrescine) for the detection of very closely spaced abasic (AP) sites (1-5 bp apart), which are poorly detected by Nfo AP endonuclease. Based on the same principle of enzyme-detection of clusters as enzyme sensitive sites, other groups have also used neutral agarose gel electrophoresis and fraction of activity released (FAR) assay, hybridization assay or plasmid nicking assay [38,39].

Finally, in an attempt of a second independent method different groups have developed a modified version of the neutral single cell gel electrophoresis (Comet assay) using again repair enzymes as damage probes [19,40]. Since its early introduction in 1984 [41] and later in its alkaline version in 1988 [42], the single-cell gel electrophoresis (SCGE) or Comet assay a modified version of the microgel electrophoresis, has been widely used for the detection of low levels of various types of DNA lesions including SSBs, DSBs and oxidized bases as reviewed in [43,44]. The Comet or microgel assay under neutral running conditions has been used for the detection of DSBs in a variety of cells including lymphocytes [4548]. To our knowledge there are very limited data on the use of the neutral Comet [40,47] or microgel assay [15,19] for the detection of non-DSB clustered DNA lesions using different repair enzymes as enzymatic probes. In all cases, incomplete cleavage of lesions by the repair enzymes can lead to a detection of only a fraction of the clusters (Fig. 1). This issue becomes very important especially in the case of high-LET radiations where the density of the lesions is expected to be very high [49]. The clusters described in Fig. 1 are an idealized form a simple cluster. In reality and particularly in the case of high-LET, one would expect a complex DNA lesion, for example a DSB with 5-10 additional surrounding lesions [20]. Therefore each cluster detected by the enzyme is only a fraction of the lesions participating in the cluster.

Table 1. The different *E-coli* or human repair enzymes and their substrates used in the detection of oxidative clustered DNA lesions [7,50)].

Repair enzymes used as damage probes	**Substrates**
E. coli Nfo protein or human hAPE1 (Endonuclease IV)	Abasic: Several types of abasic sites including oxidized abasic sites, abasic sites modified with alkoxyamines and DNA containing urea residues
E.coli Fpg protein or hOGG1 protein (DNA glycosylase) Associated lyase activity	Oxypurines: FapyAdenine, FapyGuanine, C8-oxoGuanine, some abasic sites, C8-oxoAdenine and to a lesser extent, other modified purines.
E.coli or human Nth1 protein (Endonuclease III) Associated lyase activity	Oxypyrimidines: Thymine residues damaged by ring saturation, fragmentation, or ring contraction including thymine glycol and uracil residues.

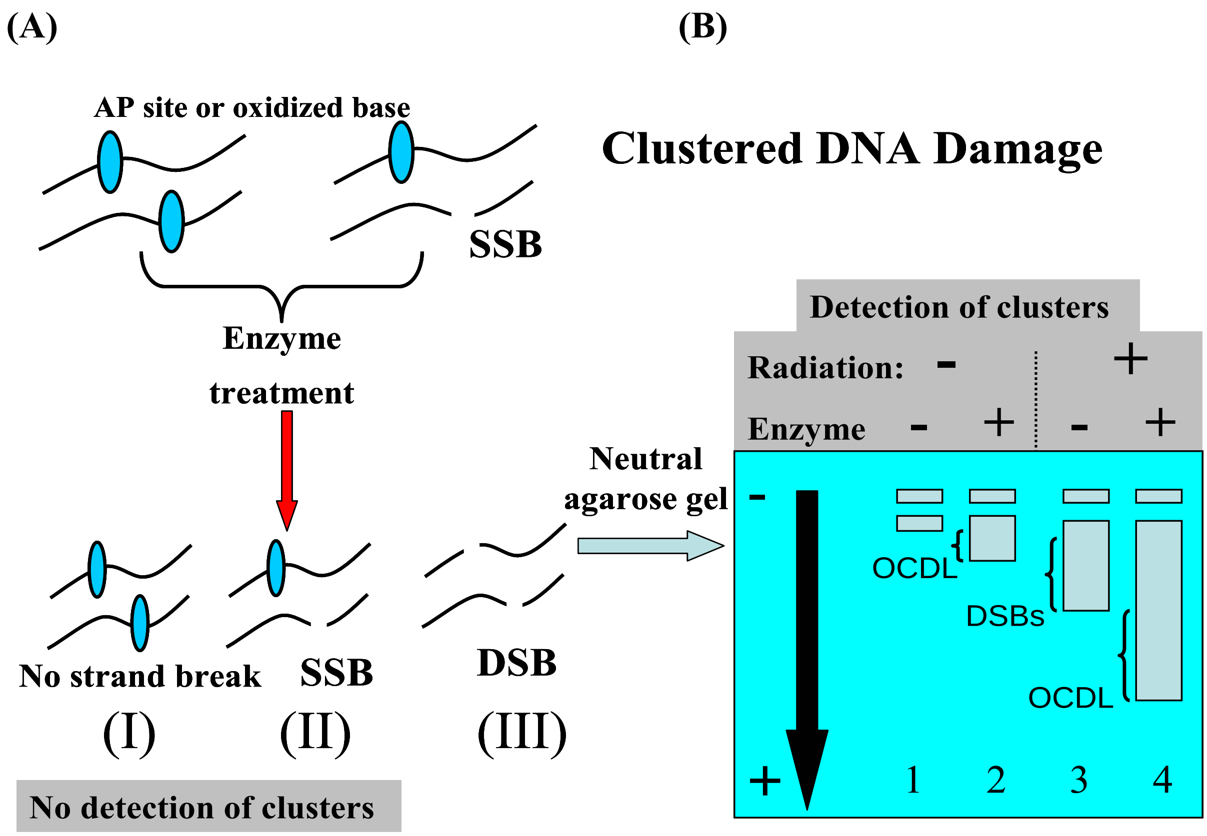

Fig. 1 Detection of bistranded clustered DNA lesions (oxidative clustered DNA lesions, OCDL) using neutral agarose gel electrophoresis. **(A)** Principles of detection using a repair enzyme for two representative type of clusters consisting of a set of bistranded base lesions or a base lesion and a single strand break, SSB. As shown in pathways I and II, incomplete cleavage of both lesions by the repair enzyme will lead to no detection of the cluster. In the case of cleavage of both lesions by the enzyme (pathway III) and induction of a DSB, detection of the cluster occurs. **(B)** Detection of clusters using neutral agarose gel electrophoresis. Genomic DNA (T7 or λ) or human DNA in agarose plugs can be subjected to agarose gel electrophoresis (constant or pulsed field according to the DNA size) and with application of number average length analysis (NALA) the prompt DSBs and OCDL can be measured in the same gel. Comparison of lanes 1 and 2 will provide the endogenous (non-irradiated) OCDL yields which usually are expected to be low. Comparison of lanes 3 and 4 provides the OCDL yields for the irradiated samples. In this case mush higher levels of OCDL are expected. Comparison of lanes 3 and 1 will provide the yield of prompt DSBs induced directly by radiation. In every case the treatment with the enzyme results in an additional fragmentation i.e., DSBs which are equal to the number of clusters.

INDUCTION OF CLUSTERED DNA LESIONS BY HIGH-LET RADIATIONS

Theoretical considerations suggest that, in addition to isolated lesions, low-LET radiation can create clusters with as many as 10 lesions 51). High-LET radiation is capable of producing damage of even greater complexity, i.e., up to 25 lesions per cluster 51). Space travel encompasses exposure to a broad spectrum of radiation ranging from the infrared to galactic cosmic rays. The major component of galactic cosmic rays is the highly charged, energetic (HZE) particles ranging from energetic protons to iron nuclei with energies ranging upwards to 1 GeV/nucleon; although of lower fluence than protons, large contributions to dose arise from Fe and other very high LET nuclei, such as N, Si, etc. 52). Estimates of space radiation health risk and the development of efficient countermeasures are key issues for manned space exploration. Accurate risk calculations require a detailed investigation of both the physical aspects (patterns of energy deposition at the molecular/cellular level) and the biological response to high LET particles. The uncertainties in radiation risk assessment for deep-space missions are between 400 and 1,500% 53). These uncertainties are largely due to the lack of information on the biologic response to HZE particles relative to the more extensively studied biological response to low LET radiation. Late effects of high-LET radiation are arguably the health risk not only for the human space exploration but also for increasing number of cancer patients treated by heavy-ion therapy including young adults and children.

Measurement of DSBs for high LET radiations has been controversial 54). In addition, very limited data exists for the efficacy of high-LET particles to induce OCDL in human cells 10,34,55,56). Many early studies on DSB induction by radiation of different qualities have shown an increased yield of DSBs with increasing LET in non-cellular systems 57) while other later studies have shown a decline of DSBs with increasing LET for SV40 DNA under high radio-quenching conditions [58] or plasmid DNA [39] irradiated in radio-quenching conditions mimicking the cellular chemical environment. For mammalian cells though, relative biological effectiveness (RBE) values for DSB induction have been usually found close or a little bit higher to unity [59,60]. The exclusion of smaller DNA fragments (<10 kbp) during PFGE analysis of DSBs can have a significant impact on the measurement of DSBs and OCDL for high-LET radiations [61]. Friedland *et al.* [61] using the PARTRAC Monte Carlo simulation code have shown that the DSB yield can drop significantly i.e., RBE values smaller than unity if during DSB measurements DNA fragments smaller than 10 kbp are excluded. Experimental studies have shown the importance of small DNA fragments (<200 kbp) especially in the case of high-LET charged particles [6265] due to the non-random distribution of breaks [6365]. The advantage of using NALA, especially in the case of high-LET radiations, is that this method does not require any specific knowledge of the distribution of DNA fragments (e.g., random or Poisson-distributed cleavage) [37]. Another significant parameter related to the detection of clustered DNA lesions are the heat-labeled sites within locally multiply damaged sites which are produced by radiation and are subsequently transformed into DSBs during the lysis procedure. These artifactual DSBs can consist 30-50% of the real DSBs induced by radiation depending on the lysis conditions (37°C or 50°C) [14,66]. .

In the case of non-DSB clusters the situations is clearer. A decrease has been detected for OCDL yields and for high-LET radiations compared to low LET and RBE values much lower than unity have been found by different groups using different DNA systems and methods [34,56,67,68]. Theoretical analysis and specifically Monte Carlo simulations predict that the initial yield of clusters other than the DSB tends to decrease with increasing particle LET, which is consistent with experimental observations [69]. In addition, several *in vitro* studies using different

DNAs in radio-quenching solutions indicate a similar decrease of clusters with increasing LET summarized also in Table 2 [34,68,70,71]. This decrease could be the result of an actual decline in the induced oxybase lesions [72] and SSBs [39,73] for high LET charged particles associated with decreased formation of radicals in the solution by ions of higher LET [74], or an inability of the methods to detect these highly dense clusters. By the last comment, we refer to the already established compromised ability of repair enzymes to detect and cleave very closely spaced DNA lesions [29,33,75]. Earlier studies using enzymatic (Fpg and/or Nth) or cell extract treatment of γ- or α-irradiated plasmid DNA under cell mimetic conditions suggest an increase of the contribution of base lesions to clustered DNA damage with LET [76]. Finally and related to all the gel electrophoresis based methods for detection of clusters, an important issue that should be mentioned here is that even if the enzymes cleave the resulting DNA fragments (<1 kbp) will remain undetected under the most current electrophoresis separation regimens applied as discussed above. Two general trends can be mentioned: i) For DSBs, a small dependence to LET with a tendency to increase at least for LET values 1-150 keV/μm, for non-DSB clusters a decline with LET and a linear dependence on dose for both DSBs and OCDL and ii) In most cases, non-DSB cluster yields (Fpg-, EndoIII- and EndoIV-clusters) tend to be lower than the corresponding yields of prompt DSBs for high-LET radiations. For low-LET radiations, the non-DSB clusters tend to be higher compared to prompt DSBs (Table 2).

Table 2. Data available on the dependence of non-DSB clustered DNA damage to LET. The yields of DSBs and non-DSB clusters have been included. The different types of clusters are presented according to the type of repair enzyme used for the detection i.e., Fpg-, EndoIII and EndoIV-clusters. Yields are presented as lesions/Gbp/Gy.

LET (keV/µm)	**Radiation**	**DSBs /Gbp/Gy**	**Fpg- /Gbp/Gy**	**EndoIII- /Gbp/Gy**	**EndoIV- /Gbp/Gy**	**Model System**
1	^{60}Co γ-rays	15		48		Plasmid pMSG-CAT
110	^{238}Pu α-particles	25		15		10 mM Tris
						Ref. 71)
1	^{60}Co γ-rays	7.1		29.9		Plasmid pMSG-CAT
110	^{238}Pu α-particles	15		5		200 mM Tris
						Ref. 71)
0.3	^{137}Cs γ-rays	0.81 x 10^3		2.35 x 10^3		Plasmid pEC
97	^{244}Cm α-particles	071 x 10^3				10 mM phosphate buffer
145	^{56}Fe	0.31 x 10^3		0.35 x 10^3		Ref. 77)
1440	^{197}Au	0.25 x 10^3				
0.225	^{1}H	9.56 x 10^3	6.67 x 10^3		4.09 x 10^3	T7 DNA in PBS
0.3	^{137}Cs γ-rays	3.78 x 10^3				Ref. 68)
2	X-ray	5.62 x 10^3	10.05 x 10^3		5.34 x 10^3	
12.97	^{12}C	3.72 x 10^3	4.37 x 10^3		2.04 x 10^3	
50.32	^{28}Si	3.57 x 10^3	2.85 x 10^3		1.27 x 10^3	
107.7	^{48}Ti	2.81 x 10^3	2.42 x 10^3		1.30 x 10^3	
150.4	^{56}Fe	1.95 x 10^3	2.35 x 10^3		1.22 x 10^3	
0.3	^{137}Cs γ-rays	11.9	10.68	11.87	9.5	Human monocytes
148	^{56}Fe	10.9	7.12	8.54	5.5	Ref. 34)

Hada and Sutherland [68] and Tsao *et al.* [34] have also shown a prevalence of Fpg-clusters to Endo IV (Nfo)-or EndoIII-clusters for T7 or human DNA irradiated with ^{56}Fe ions under radio-quenching conditions (Table 2). As mentioned earlier, another effect that has to be taken into consideration is the conversion of heat labile sites to strand breaks [78]. It is a possible source of overestimation of DSBs and non-DSB clusters and, especially in the case of γ rays, the artifactual conversion of heat labile sites into DSB can result in the underestimation of RBE values for Fe ions [59]. In general, and as also stated by Boucher *et al.* [79], the role of artifactual DNA oxidation is very important but based on the above different group data, its role in the case of high-LET radiations is expected to be minimum. Finally as shown in Table 1, the overlapping specificity of the different repair enzymes is another potential source of overestimation in the measurement of total number of non-DSB clusters that can reach in some cases even a factor of two [40].

CONCLUDING COMMENTS

Although a significant amount of data has been produced on the efficiency of high-LET radiations to induce clustered DNA damage, still many questions remain unanswered. Significant deviations appear between theoretical and experimental data. Therefore, we believe that the primary objectives of the future studies should be to i) provide analytical, quantitative, and theoretical information on the induction and processing of different patterns of complex DNA damage (DSB and non-DSB clustered DNA lesions) produced directly and indirectly (bystander effects) by high-LET radiations *in vivo* and *in vitro* and ii) measure the biological effectiveness of high-LET radiations (like space HZE particles or α-particles) for the induction of chromosome damage and apoptosis *in vivo* and *in vitro* and correlate these endpoints with the levels of complex DNA damage detected in each case, and finally iii) improve the experimental detection methods for including the very small DNA fragments expected to be induced in the case of high-LET radiations. A better knowledge of the induction and repair of complex DNA damage is essential for the calculations of the risk factors of high LET radiations to induce carcinogenesis (as in the case of radon-gas emitted α-particles, heavy-ion therapy or space radiation).

ACKNOWLEDGEMENTS

This work has been supported by funds provided to Dr. Georgakilas by the Biology Department of East Carolina University, a Research/Creative Activity Grant and a College Research Award to A. Georgakilas (East Carolina University).

REFERENCES

1. Preston, D. L. and Piece, D. A. (2003) Studies of mortality of atomic bomb survivors. Report 13: Solid cancer and noncancer disease mortality: 1950-1997. Radiat. Res. **160**: 381-407.
2. Ward, J. F. (1981) Some biochemical consequences of the spatial distribution of ionizing radiation produced free radicals. Radiat. Res. **86**: 185-195.
3. Ward, J. F. (1985) Biochemistry of DNA lesions. Radiat. Res. **104**: S103-S111.
4. Ward, J. F. (1994) The complexity of DNA damage: relevance to biological consequences. Int. J. Radiat. Biol. **66**: 427-432.
5. Wallace, S. S. (1998) Enzymatic Processing of Radiation-Induced Free Radical Damage in DNA. Radiat. Res. **150 (suppl.)**: S60-S79.
6. Goodhead, D. T., Thacker, J. and Cox, R. (1993) Effects of radiations of different qualities on cells: Molecular mechanism of damage and repair. Int. J. Radiat. Biol. **63**(543-556).
7. Sutherland, B. M., Bennett, P. V., Sidorkina, O. and Laval, J. (2000) Clustered Damages and Total Lesions Induced in DNA by Ionizing Radiation: Oxidized Bases and Strand Breaks. Biochemistry **39**: 8026-8031.
8. Gollapalle, E., Wong, R., Adetolu, R., Tsao, D., Francisco, D., Sigounas, G. and Georgakilas, A. G. (2007) Detection of oxidative clustered DNA lesions in X-irradiated mouse skin tissues and human MCF-7 breast cancer cells. Radiat. Res. **167**: 207-216.
9. Goodhead, D. T. (1994) Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. Int. J. Radiat. Biol. **65**: 7-17.

10. Sutherland, B., Bennett, P. V., Sidorkina, O. and Laval, J. (2000) DNA Damage Clusters Induced by Ionizing Radiation in Isolated DNA and in Human Cells. Proc. Natl. Acad. Sci. USA **97**: 103-108.

11. Povirk, L. F. and Houlgrave, C. W. (1988) Effect of apurinic/apyrimidinic endonucleases and polyamines on DNA treated with bleomycin and neocarzinostatin: Specific formation and cleavage at closely opposed lesions in complementary strands. Biochemistry **27**: 3850-3857.

12. Regulus, P., Duroux, B., Bayle, P. A., Favier, A., Cadet, J. and Ravanat, J. L. (2007) Oxidation of the sugar moiety of DNA by ionizing radiation or bleomycin could induce the formation of a cluster DNA lesion. Proc. Natl. Acad. Sci. USA **104**: 14032-14037.

13. Sutherland, B. M., Bennett, P. V., Sutherland, J. C. and Laval, J. (2002) Clustered DNA damages induced by X-rays in human cells. Radiat. Res. **157**: 611-616.

14. Gulston, M., C., d. L., Jenner, T., Davis, E. and O'Neill, P. (2004) Processing of clustered DNA damage generates additional double-strand breaks in mammialian cells post-irradiation. Nucleic Acids Res. **32**: 1602-1609.

15. Yang, N., Chaudhry, M. A. and Wallace, S. S. (2006) Base excision repair by hNTH1 and hOGG1: a two edged sword in the processing of DNA damage in gamma-irradiated human cells. DNA Repair **5**: 43-51.

16. Dugle, D., Gillespie, C. and Chapman, J. D. (1976) DNA strand breaks, repair and survival in X-irradiated mammalian cells. Proc. Natl. Acad. Sci. U. S. A. **73**(809-812).

17. Ahnstrom, G. and Bryant, P. E. (1982) DNA double-strand breaks generated by the repair of X-ray damage in Chinese hamster cells. Int. J. Radiat. Biol. **41**: 671-676.

18. Bonura, T. and Smith, K. C. (1975) Enzymatic production of deoxyribonucleic acid double-strand breaks after ultraviolet irradiation of *Escherichia coli* K12. J. Bacteriol. **121**: 511-517.

19. Blaisdell, J. O. and Wallace, S. (2001) Abortive base-excision repair of radiation-induced clustered DNA lesions in Escherichia coli. Proc. Natl. Acad. Sci. USA **98**: 7426-30.

20. Georgakilas, A. G. (2008) Processing of DNA damage clusters in human cells: Current status of knowledge Mol. Biosyst. **4**: 30-35.

21. Bennett, P. V., Cuomo, N. L., Paul, S., Tafrov, S. T. and Sutherland, B. M. (2005) Endogenous DNA damage clusters in human skin, 3-D model and cultured skin cells. Free Radic. Biol. Med. **39**: 832-839.

22. Lindahl, T. and Wood, R. D. (1999) Quality control of DNA repair. Science **286**: 1897-1905.

23. Pearson, C. G., Shikazono, N., Thacker, J. and O'Neill, P. (2004) Enhanced mutagenic potential of 8-oxo-7,8-dihydroguanine when present within a clustered DNA damage site. Nucleic Acids Res. **32**: 263-270.

24. Malyarchuk, S., Brame, K. L., Youngblood, R., Shi, R. and Harrison, L. (2004) Two clustered 8-oxo-7,8-dihydroguanine (8-oxodG) lesions increase the point mutation frequency of 8-oxodG, but do not result in double strand breaks or deletions in Escherichia coli. Nucleic Acids Res. **32**: 5721-5731.

25. Cunniffe, S. M., Lomax, M. E. and O'Neill, P. (2007) An AP site can protect against the mutagenic potential of 8-oxoG when present within a tandem clustered site in E. coli. DNA Repair **doi:10.1016/j.dnarep.2007.07.003**.

26. Chaudhry, M. A. and Weinfeld, M. (1997) Reactivity of human apurinic/apyrimidinic endonuclease and *Escherichia coli* exonuclease III with bistranded abasic sites in DNA. J. Biol. Chem. **272**(25): 15650-15655.

27. Harrison, L., Hatahet, Z. and Wallace, S. (1999) *In Vitro* Repair of Synthetic Ionizing Radiation-induced Multiply Damaged DNA Sites. J. Mol. Biol. **290**: 667-684.

28. McKenzie, A. A. and Strauss, P. R. (2001) Oligonucleotides with bistranded abasic sites interfere with substrate binding and catalysis by human apurinic/apyrimidinic endonuclease. Biochemistry **40**: 13254-13261.

29. David-Cordonnier, M. H., Cunniffe, S. M. T., Hickson, I. D. and O'Neill, P. (2002) Efficiency of incision of an AP site within clustered DNA damage by the major human AP endonuclease. Biochemistry **41**: 634-642.

30. Georgakilas, A. G., Bennett, P. V. and Sutherland, B. M. (2002) High efficiency detection of bistranded abasic clusters in g-irradiated DNA by putrescine. Nucleic Acids Res. **30**: 2800-2808.

31. Lomax, M. E., Cunniffe, S. and O'Neill, P. (2004) 8-OxoG retards the activity of the ligase III/XRCC1 complex during the repair of a single-strand break, when present within a clustered DNA damage site. DNA Repair **3**: 289-299.

32. Eot-Houllier, G., Eon-Marchais, S., Gasparutto, D. and Sage, E. (2005) Processing of a complex multiply damaged DNA site by human cell extracts and purified repair proteins. Nucleic Acids Res. **33**: 260-271.

33. Georgakilas, A. G., Bennett, P. V., Wilson III, D. M. and Sutherland, B. M. (2004) Processing of bistranded abasic DNA clusters in gamma-irradiated human hematopoietic cells. Nucleic Acids Res. **32**: 5609-5620.

34. Tsao, D., Kalogerinis, P., Tabrizi, I., Dingfelder, M., Stewart, R. D. and Georgakilas, A. G. (2007) Induction and Processing of clustered DNA lesions in human monocytes exposed to low doses of HZE ^{56}Fe particles. Radiat. Res. **168**: 87-97.

35. Sutherland, B. M., Georgakilas, A. G., Bennett, P. V., Laval, J. and Sutherland, J. C. (2003) Quantifying clustered DNA damage induction and repair by gel electrophoresis, electronic imaging and number average length analysis. Mutat. Res. (Review) **531**: 93-107.

36. Terato, H. and Ide, H. (2004) Clustered DNA damage induced by heavy ion particles. Biol. Sci. Space **18**: 206-215.

37. Sutherland, B. M., Bennett, P. V., Georgakilas, A. G. and Sutherland, J. C. (2003) Evaluation of Number Average Length Analysis In Quantifying Double Strand Breaks in Genomic DNAs. Biochemistry **42**: 3375-3384.

38. Gulston, M., Fulford, J., Jenner, T., de Lara, C. and O'Neill, P. (2002) Clustered DNA damage induced by g radiation in human fibroblasts (HF 19), hamster (V79-4) cells and plasmid DNA is revealed as Fpg and Nth sensitive sites. Nucleic Acids Res. **30**: 3464-3472.

39. Leloup, C., Garty, G., Assaf, G., Cristovao, A., Breskin, A., Chechik, R., Shchemelinin, S., Paz-Elizur, T., Livneh, Z., Schulte, R. W., Bashkirov, V., Milligan, J. R. and Grosswendt, B. (2005) Evaluation of lesion clustering in irradiated plasmid DNA. Int. J. Radiat. Biol. **81**: 41-54.

40. Holt, S. M. and Georgakilas, A. G. (2007) Detection of complex DNA damage in γ-irradiated acute lymphoblastic leukemia pre-B NALM-6 cells. Radiat. Res. **168**: 527-534.

41. Ostling, O. and Johanson, K. J. (1984) Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem. Biophys. Res. Commun. **123**: 291-298.

42. Singh, N. P., McCoy, M. T., Tice, R. R. and Schneider, E. L. (1998) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. **175**: 184-191.

43. Olive, P. L. and Banath, J. P. (2006) The comet assay: a method to measure DNA damage in individual cells. Nat. Protoc. **1**: 23-29.

44. Collins, A. R. (2004) The Comet assay for DNA damage and repair: Principles, applications and limitations (Review). Molecular Biotechnology **26**: 249-261.

45. Trenz, K., Schutz, P. and Speit, G. (2005) Radiosensitivity of lymphoblastoid cell lines with a heterozygous BRCA1 mutation is not detected by the comet assay and pulsed field gel electrophoresis. Mutagenesis **20**: 131-137.

46. Yang, N., Galick, H. and Wallace, S. S. (2004) Attempted base excision repair of ionizing radiation damage in human lymphoblastoid cells produces lethal and mutagenic double strand breaks. DNA Repair **3**: 1323-1334.

47. Angelis, K. J., Dusinska, M. and Collins, A. R. (1999) Single cell gel electrophoresis: detection of DNA damage at different levels of sensitivity. Electrophoresis **20**: 2133-2138.

48. Olive, P. L. and Banath, J. P. (1993) Detection of DNA double-strand breaks through the cell cycle after exposure to X-rays, bleomycin, etoposide and 125IdUrd. Int. J. Rad. Biol. **64**: 349-358.

49. Nikjoo, H., O' Neill, P., Wilson, E. W. and Goodhead, D. (2001) Computational approach for determing the spectrum of DNA damage induced by ionizing radiation. Radiat. Res. **156**: 577-583.

50. Wilson , D. M., Sofinowski, T. M. and McNeill, D. R. (2003) Repair mechanisms for oxidative DNA damage. Frontiers in Bioscience **8**: 963-981.

51. Semenenko, V. A. and Stewart, R. D. (2004) A fast Monte Carlo algorithm to simulate the spectrum of DNA damages formed by ionizing radiation. Radiat. Res. **161**: 451-457.

52. Ponomarev, A. L., Cucinotta, F. A., Sachs, R. K. and Brenner, D. J. (2001) Monte Carlo predictions of DNA fragment-size distributions for large sizes after HZE particle irradiation. Physica Medica **XVII**: 153-156.

53. NASA (1998). Strategic program plan for space radiation health research. Washington,DC, NASA.

54. Prise, K. M., Pinto, M., Newman, H. C. and Michael, B. D. (2001) A review of studies of ionizing radiation-induced double-strand break clustering. Radiat Res **156**: 572-576.

55. Terato, H., Tanaka, R., Nakaarai, Y., Furusawa, Y. and Ide, H. (2004) Analysis of DNA damage generated by high-energy particles. Nucleic Acids Symp. Ser. (Oxf). **48**: 145-146.

56. Terato, H., Tanaka, R., Nakaarai, Y., Hirayama, R., Furusawa, Y. and Ide, H. (2007) Analysis of complex DNA lesions generated by heavy ion beams. Nucleic Acids Symp. Ser. (Oxf) **51**(1): 221-222.

57. Neary, G. J., Horgan, V. J., Bance, D. A. and Stretch, A. (1972) Further data on DNA strand breakage by various radiation qualities. Int. J. Radiat. Biol. **22**: 525-537.

58. Taucher-Scholz, G. and Kraft, G. (1999) Influence of radiation quality on the yield of DNA strand breaks in SV40 DNA irradiated in solution. Radiat. Res. **151**: 595-604.

59. Belli, M., Campa, A., Dini, V., Esposito, G., Furusawa, Y., Simone, G., Sorrentino, E. and Tabocchini, M. A. (2006) DNA fragmentation induced in human fibroblasts by accelerated ^{56}Fe ions of differing energies. Radiat Res **165**: 713-720.

60. Prise, K. M., Ahnstrom, G., Belli, M., Carlsson, J., Frankenberg, D., Kiefer, J., Lobrich, M., Michael, B. D., Nygren, J., Simone, G. and Stenerlow, B. (1998) A review of dsb induction data for varying quality radiation. Int. J. Radiat. Biol. **74**: 173-184.

61. Friedland, W., Dingfelder, M., Jacob, P. and Paretzke, H. G. (2005) Calculated DNA double-strand break and fragmentation yields after irradiation with He ions. Radiat. Phys. Chem. **72**: 279-286.

62. Ponomarev, A. L. and Cucinotta, F. A. (2006) Chromatin loops are responsible for higher counts of small DNA fragments induced by high-LET radiation, while chromosomal domains do not affect the fragment sizes. Int. J. Radiat. Biol. **82**: 293-305.

63. Friedland, W., Jacob, P., Paretzke, H. G. and Stork, T. (1998) Monte Carlo simulation of the production of short DNA fragments by low-linear energy transfer radiation using higher-order DNA models. Radiat. Res. **150**: 170-182.

64. Lobrich, M., Cooper, P. K. and Rydberg, B. (1996) Non-random distribution of DNA double-strand breaks induced by particle irradiation. Int. J. Radiat. Biol. **70**: 493-503.

65. Rydberg, B. (1996) Clusters of DNA Damage Induced by Ionizing Radiation: Formation of Short DNA Fragments. II. Experimental Detection. Radiation Research **145**: 200-209.

66. Rydberg, B., ouml and rn (2000) Radiation-Induced Heat-Labile Sites that Convert into DNA Double-Strand Breaks. Radiation Research **153**(6): 805-812.

67. Sutherland, B. M., Bennett, P., Georgakilas, A. G. and Hada, M. (2003). Bistranded DNA Damage Clusters Induced by Low LET Radiation and Heavy Charged Particles: Formation and Repair. 14th Annual NASA Space Radiation Investigator's Workshop, April 27-30, Houston, Texas, DOE/NASA.

68. Hada, M. and Sutherland, B. M. (2006) Spectrum of complex DNA damages depends on the incident radiation. Radiat. Res. **165**: 223-230.

69. Semenenko, V. A. and Stewart, R. D. (2006) Fast Monte Carlo simulation of DNA damage formed by electrons and light ions. Phys. Med. Biol. **51**: 1693-1706.

70. Milligan, J. R., Aguilera, J. A., Nguyen, T. T., Paglinawan, R. A. and Ward, J. F. (2000) DNA strand-break yields after post-irradiation incubation with base excision repair endonucleases implicate hydroxyl radical pairs in double-strand break formation. Int. J. Radiat. Biol. **76**: 1475-83.

71. Prise, K. M., Pullar, C. H. and Michael, B. D. (1999) A study of endonuclease III-sensitive sites in irradiated DNA: detection of alpha-particle-induced oxidative damage. Carcinogenesis **20**: 905-909.

72. Pouget, J.-P., Ravanat, J.-L., Douki, T., Richard, M.-J. and Cadet, J. (1999) Measurement of DNA base damage in cells exposed to low doses of gamma-radiation: comparison between the HPLC-EC and comet assays. Int. J. Radiat. Biol. **75**: 51-58.

73. Georgakilas, A. G., Haveles, K. S., Sophianopoulou, V., Sakelliou, L., Zarris, G. and Sideris, E. G. (2000) alpha-Particle-induced changes on the stability and size of DNA. Radiat. Res. **153**: 258-262.

74. Moritake, T., Tsuboi, K., Anzai, K., Ozawa, T., Ando, K. and Nose, T. (2003) ESR spin trapping of hydroxyl radicals in aqueous solution irradiated with high-LET carbon-ion beams Radiat. Res. **159**: 670-675.

75. David-Cordonnier, M. H., Boiteux, S., and and O'Neill, P. (2001) Efficiency of excision of 8-oxo-guanine within DNA clustered damage by XRS5 nuclear extracts and purified human OGG1 protein. Biochemistry **40**: 11811-11818.

76. Jenner, T. J., Fulford, J. and Neill, P. (2001) Contribution of Base Lesions to Radiation-Induced Clustered DNA Damage: Implication for Models of Radiation Response. Radiat. Res. **156**(5): 590-593.

77. Milligan, J. R., Aguilera, J. A., Paglinawan, R. A., Ward, J. F. and Limoli, C. L. (2001) DNA strand break yields after post-high LET irradiation incubation with endonuclease-III and evidence for hydroxyl radical clustering. Int. J. Radiat. Biol. **77**: 155-164.

78. Stenerlow, B., Karlsson, K. H., Cooper, B. and Rydberg, B. (2003) Measurement of prompt DNA double-strand breaks in mammalian cells without including heat-labile sites: results for cells deficient in nonhomologous end joining. Radiat. Res. **159**: 502-510.

79. Boucher, D., Testard, I. and Averbeck, D. (2006) Low levels of clustered oxidative DNA damage induced at low and high LET irradiation in mammalian cells. Radiat. Environ. Biophys. **45**: 267-276.