Detection of clustered DNA lesions: Biological and clinical applications

Alexandros Georgakilas

Abstract

Humans are daily exposed to background radiation and various sources of oxidative stress. My research has focused in the last 12 years on the effects of ionizing radiation on DNA, which is considered as the key target of radiation in the cell. Ionizing radiation and endogenous cellular oxidative stress can also induce closely spaced oxidatively induced DNA lesions called “clusters” of DNA damage or locally multiply damage sites, as first introduced by John Ward. I am now interested in the repair mechanisms of clustered DNA damage, which is considered as the most difficult for the cell to repair. A main part of my research is devoted to evaluating the role of clustered DNA damage in the promotion of carcinogenesis in vitro and in vivo. Currently in my laboratory, there are two main ongoing projects. (1) Study of the role of BRCA1 and DNA-dependent protein kinase catalytic subunit repair proteins in the processing of clustered DNA damage in human cancer cells. For this project, we use several tumor cell lines, such as breast cancer cell lines MCF-7 and HCC1937 (BRCA1 deficient) and human glioblastoma cells MO59J/K; and (2) Possible use of DNA damage clusters as novel cancer biomarkers for prognostic and therapeutic applications related to modulation of oxidative stress. In this project human tumor and mice tissues are being used.

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Key words: DNA damage clusters; DNA repair; Oxidative stress; Cancer; Biomarkers

INTRODUCTION AND EDUCATIONAL EXPERIENCE

Dr. Alexandros Georgakilas is an Associate Professor of...
Biology in the Department of Biology at East Carolina University (ECU), Greenville, NC, USA. He received his bachelor’s degree with honors in physics at the University of Athens, Greece in 1992 (Figure 1). He pursued postgraduate studies in molecular and cellular biology at the Institute of Biology, NCSR “Demokritos” in 1993. Following postgraduate studies, Dr. Georgakilas received his doctorate with honors in radiation biology on the “Effects of α-particles radiation on mammalian DNA” in the Department of Biology at the University of Athens, Greece in 1998. Subsequently, Dr. Georgakilas was a postdoctoral research associate in the Institute of Biology, NCSR “Demokritos” (1999-2000) and in the Department of Physics, Health and Medical Physics Group, University of Athens, as well as Aretaion Oncological Hospital in Athens, Greece (2000-2001). Additionally, at the Brookhaven National Laboratory, Dr. Georgakilas completed postdoctoral research as an associate in the Biology Department from 2001-2003 under the supervision of Dr. Betsy Sutherland. He was supported by Fellowship awards, including the ESRB 1996 Fellowship, European Radiation Research Society Fellowship, NATO Fellowship, Gordon Fellowship, and Post-Doctorate Fellowship of the Greek Secretariat of Technology. He has also received awards such as the Young Investigator Travel Award from Radiation Research Society, Radiation Research Society SIT Award, ECU Thomas Harriot College Research Award, and Terashima Award from the Japan Radiation Research Society. Dr. Georgakilas has participated and presented to many international meetings, numerous national meetings of Greek and US Scientific Societies, has more than forty (40) publications in peer-reviewed scientific journals, and has many publications in books and conference proceedings from international meetings.

ACADEMIC STRATEGY AND GOALS

Over recent years, Dr. Georgakilas’ research group at ECU has developed a reliable methodology for the study of clustered DNA lesions at the cellular or tissue level using human or bacterial DNA repair enzymes based on the pioneering work of Dr. Sutherland B[11]. There are limited data on the use of the DNA damage molecular assays for the detection of bi-stranded non-double strand break (DSB) oxidative clustered DNA lesions (OCDLs) in vitro or in vivo. Dr. Georgakilas’ work has provided a novel adaptation of neutral single cell gel electrophoresis (comet assay) or pulsed field gel electrophoresis to measure these unique types of lesion and their repair. Thus far, Dr. Georgakilas’ research has contributed to the identification of novel DNA repair pathways for OCDLs, as well as uncovering different strategies that these mutagenic and repair resistant lesions are processed by the cell or human tissue[3]. In this way, meaningful insights can be discovered for the biological and clinical application of DNA damage clusters[4].

ACADEMIC ACHIEVEMENTS

The following contributions highlight Dr. Georgakilas’ activities in the field of oxidative stress and DNA damage repair.

Detection of OCDLs in vivo: potential clinical applications

Dr. Georgakilas and his team through collaborations with various elite research groups (ECU, NCI) have taken advantage of the properties of human repair enzymes OGG1 (oxidized purines), EndoIII (oxidized pyrimidines) and APE1 (abasic sites) in order to detect a variety of bi-stranded OCDLs in human and mouse tissues, using adaptations of DNA gel electrophoresis[4-8]. The concepts in DNA cluster quantification are simple, but their execution is technically and analytically demanding. Dr. Georgakilas’ group, using novel adaptations of DNA gel electrophoresis, has been able to provide to the scientific community relatively easy and reliable methodologies for the detection of OCDLs in a variety of cell and tissues[8-11]. In early 2007, Gollapalle et al[12] showed for the first time in vivo that X-rays can induce a radiation-delayed effect in mice several weeks after the initial irradiation. In addition, they showed evidence of bystander or so-called distal effects[12]. In a later study, Nowsheen et al[13] showed that, in the majority of human tumor cases examined, there were higher levels of OCDLs in tumors compared with normal tissues. These data suggest for the first time the importance of endogenous non-DSB clusters in human cancer and their potential use as cancer biomarkers. Finally, Redon et al[10] at NCI, have published a seminal paper in Proceedings of the National Academy of Sciences USA, targeting the induction of OCDLs and DSBs in tumor-bearing mice. Specifically, they showed for the first time, that tumors induce via a cytokine CCL2-mechanism clustered DNA damage in distant tissues, with proliferative organs being more susceptible to DNA damage induction.

OCDL repair pathways in human cells: biological significance

Dr. Georgakilas’ group has been able to show the involvement of specific DNA repair pathways and proteins in the processing of OCDLs after exposure to radiotherapy-relevant doses of ionizing radiation. Peddi et al[13], Georgakilas[14] and Peddi et al[15] showed for the first time the involvement of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) in the processing of non-DSB clusters. DNA-PKcs plays an important role during the processing of DSBs, but it has also been shown that DNA-PKcs deficiency similar to that in human tumors can lead to significant accumulation of OCDLs in a variety of human normal and malignant cells. In another study, Holt et al[16] showed that MSH2 (mismatch repair protein) is involved in the repair of OCDLs. MSH2 mutations have been identified in acute lymphoblastic leukemia (ALL), as well as in other types of cancer. In that study, the research model involved two precursor B (pre-B) ALL human cell lines, NALM-6, homozygous null for MSH2, and wild-type 697 cells. Using a modified version of neutral and alkaline single cell gel electrophoresis with Escherichia coli repair enzymes as damage
probes, the processing capacity of single-strand breaks, DSBs and OCDLs was assessed in NALM-6 and 697 cells exposed to a radiotherapy-relevant gamma-ray dose of 5 Gy. It has been suggested that MSH2 is probably involved in the processing of the biologically significant clustered DNA damage, as well as apoptosis induced by ionizing radiation. Hair et al[17] have shown the enhancement of chromosomal instability through BRCA1-based DNA repair defects. These results further support the biological significance of repair-resistant clustered DNA damage leading to chromosomal instability. The current results combined with previous findings[18] enhance the potential association of OCDLs with breast cancer development, especially in the case of BRCA1 deficiency leading to the survival of breast cells carrying a high load of unrepaired DNA damage clusters. In a recent study, Baird et al[8] showed that mild hypothermia (30°C) can modulate in a beneficial way the repair and toxic effects of clustered DNA lesions ex vivo.

CONCLUSION

Human cells in tissues and organs are continuously subjected to high oxidative stress and reactive oxygen species on a daily basis. This free radical attack can have exogenous or endogenous (intracellular) origin. The cells withstand and counteract this occurrence by the use of several and different defense mechanisms, ranging from free radical scavengers such as vitamins C and E and antioxidant enzymes such as catalase, superoxide dismutase and various peroxidases, to sophisticated and elaborate DNA repair mechanisms[19]. The outcome of this dynamic equilibrium is the induction of oxidative DNA damage that is dangerous for the cell, i.e. complex lesions such as DSBs and other non-DSBs that generate OCDLs[2,20-24].

The accumulation of DNA damage through misrepair or incomplete repair may lead to mutagenesis, chromosomal instability, and consequently, transformation, particularly if combined with a deficient apoptotic pathway (p53 mutations) (Figure 2). In this review, we present the current status of knowledge and evidence on the mechanisms and involvement of intracellular oxidative stress and DNA damage in human malignancy evolution, and possible use of these parameters as cancer biomarkers. Overall, Dr. Georgakilas’ research has provided a better understanding of the key mechanistic aspects of repair of DNA damage clusters, and evidence of the mechanisms and involvement of intracellular oxidative stress and DNA damage in human malignancy evolution and possible use of these parameters as cancer biomarkers.
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REFERENCES

1. Sutherland BM, Bennett PV, Sidorkina O, Laval J. Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation. Proc Natl Acad Sci USA 2000; 97: 103-108

2. Sedelnikova OA, Redon CE, Dickey JS, Nakamura AJ, Georgakilas AG, Bonner WM. Role of oxidatively induced DNA lesions in human pathogenesis. Mutat Res 2010; 704: 152-159

3. Georgakilas AG. Processing of DNA damage clusters in human cells: current status of knowledge. Mol Biosyst 2008; 4: 30-35

4. Collapalle E, Wang R, Adetolu R, Tsao D, Francisco D, Sigounas G, Georgakilas AG. Detection of oxidative clustered DNA lesions in X-irradiated mouse skin tissues and human MCF-7 breast cancer cells. Radiat Res 2007; 167: 207-216

5. Nowsheen S, Wukovich RL, Aziz K, Kalogerinis PT, Richardson CC, Panayiotidis MI, Bonner WM, Sedelnikova OA, Georgakilas AG. Accumulation of oxidatively induced clustered DNA lesions in human tumor tissues. Mutat Res 2009; 674: 131-136

6. Redon CE, Dickey JS, Nakamura AJ, Kareva IG, Naf D, Nowsheen S, Kryston TB, Bonner WM, Georgakilas AG, Sedelnikova OA. Tumors induce complex DNA damage in distant proliferative tissues in vivo. Proc Natl Acad Sci USA 2010; 107: 17992-17997

7. Redon CE, Dickey JS, Nakamura AJ, Kareva IG, Naf D, Nowsheen S, Kalogerinis PK, Bonner WM, Georgakilas AG, Sedelnikova OA. Tumors induce complex DNA damage in distant proliferative tissues in vivo. Cancer Res 2010; 70: 2287

8. Baird BJ, Dickey JS, Nakamura AJ, Redon CE, Parekh P, Griko YV, Aziz K, Georgakilas AG, Bonner WM, Martin OA. Hypothermia postpones DNA damage repair in irradiated cells and protects against cell killing. Mutat Res 2011; 711: 142-149

9. Holt SM, Georgakilas AG. Detection of complex DNA damage in gamma-irradiated acute lymphoblastic leukemia Pre-b NALM-6 cells. Radiat Res 2007; 168: 527-534

10. Hada M, Georgakilas AG. Formation of clustered DNA damage after high-LET irradiation: a review. J Radiat Res (Tokyo) 2008; 49: 203-210

11. Georgakilas AG, Holt SM, Hair JM, Loftin CW. Measurement of oxidatively-induced clustered DNA lesions using a novel adaptation of single cell gel electrophoresis (comet assay). Curr Protoc Cell Biol 2010; Chapter 6: Unit 6.11.

12. Martin OA, Redon CE, Nakamura AJ, Dickey JS, Georgakilas AG, Bonner WM. Systemic DNA damage related to cancer. Cancer Res 2011; 71: 3437-3441

13. Peddi P, Francisco DC, Cecil AM, Hair JM, Panayiotidis MI, Georgakilas AG. Processing of clustered DNA damage in human breast cancer cells MCF-7 with partial DNA-PKcs deficiency. Cancer Lett 2008; 269: 174-183

14. Georgakilas AG. A possible role of repair proteins BRCA1 and DNA-PK in the processing of oxidative DNA damage. J Biochem Technol 2008; 1: 9-11

15. Peddi P, Loftin CW, Dickey JS, Hair JM, Burns KJ, Aziz K, Francisco DC, Panayiotidis MI, Sedelnikova OA, Bonner WM, Winters TA, Georgakilas AG. DNA-PKcs deficiency leads to persistence of oxidatively induced clustered DNA lesions in human tumor cells. Free Radiol Biol Med 2010; 48: 1435-1443

16. Holt SM, Scemama JL, Panayiotidis MI, Georgakilas AG. Compromised repair of clustered DNA damage in the human acute lymphoblastic leukemia MSH2-deficient NALM-6 cells. Mutat Res 2009; 674: 123-130

17. Hair JM, Terzoudi GI, Hatzi VI, Lehockey KA, Srivastava D, Wang W, Pantelias GE, Georgakilas AG. BRCA1 role in the mitigation of radiotoxicity and chromosomal instability through repair of clustered DNA lesions. Chem Biol Interact 2010; 188: 350-358

18. Georgakilas AG, Bennett PV, Wilson DM, Sutherland BM. Processing of bistranded abasic DNA clusters in gamma-irradiated human hematopoietic cells. Nucleic Acids Res 2004; 32: 5609-5620

19. Franco R, Schoneveld O, Georgakilas AG, Panayiotidis MI. Oxidative stress, DNA methylation and carcinogenesis. Cancer Lett 2008; 266: 6-11

20. Georgakilas AG, Aziz K, Ziech D, Georgakilas S, Panayiotidis MI. BRCA1 involvement in toxicological responses and human cancer etiology. Toxicol Lett 2009; 188: 77-83

21. Ziech D, Franco R, Georgakilas AG, Georgakilas S, Malamou-Mitsi V, Schoneveld O, Pappa A, Panayiotidis MI. The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. Chem Biol Interact 2010; 188: 334-339

22. Ziech D, Franco R, Pappa A, Malamou-Mitsi V, Georgakilas S, Georgakilas AG, Panayiotidis MI. The role of epigenetics in environmental and occupational carcinogenesis. Chem Biol Interact 2010; 188: 340-349

23. Kryston TB, Georgiev AB, Pissis P, Georgakilas AG. Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res 2011; 711: 193-201

24. Georgakilas AG. From chemistry of DNA damage to repair and biological significance. Comprehending the future. Mutat Res 2011; 711: 1-2

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