

Response of Lymphocytes to Radiation in Untreated Breast Cancer Patients as Detected with Three Different Genetic Assays¹

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Objective To detect the response of lymphocytes to radiation in untreated breast cancer patients with three different genetic assays. **Methods** Blood samples were collected from 25 untreated patients and 25 controls. Each blood sample was divided into two parts: one was irradiated by 3-Gy X-ray (irradiated sample), the other was not irradiated (non-irradiated sample). The radiosensitivity of lymphocytes was assessed by comet assay, cytokinesis-block micronucleus (CBMN) assay and 6-TG-resistant cells scored (TG) assay. **Results** The baseline values of micronucleated cell frequency (MCF) and micronucleus frequency (MNF) in the patients were significantly higher than those in the controls ($P < 0.01$), and 3-Gy X-ray induced genetic damage to lymphocytes in the patients increased significantly as compared with that in the controls as detected with the three genetic assays ($P < 0.01$). The proportion of radiosensitive cases in the patient group was 48% for the mean tail length (MTL), 40% for the mean tail moment (MTM), 40% for MCF, 44% for MNF, and 48% for mutation frequencies of the *hprt* gene (*Mfs-hprt*), respectively, whereas the proportion of radiosensitive cases in the control group was only 8% for all the parameters. **Conclusion** The difference in the lymphocyte radiosensitivity between the breast cancer patients and the controls is significant. Moreover, there are wide individual variations in lymphocyte radiosensitivity of patients with breast cancer. In some cases, the radiosensitivity of the same patient may be different as detected with the different assays. It is suggested that multiple assays should be used to assess the radiosensitivity of patients with breast cancer before therapy.

Key words: Micronucleus assay; Comet assay; *hprt* gene mutation; Radiosensitivity; Breast cancer

REFERENCES

1. Kizilian-Martel N, Wilkins R C, McLean J R, *et al.* (2003). Prediction of radiosensitivity by measurement of X-ray induced apoptosis in human blood using the comet assay. *Anticancer Res* **5A**, 3847-3854.
2. Dunne A L, Price M E, Mothersill C, *et al.* (2003). Relationship between clonogenic radiosensitivity, radiation-induced apoptosis and DNA damage/repair in human colon cancer cells. *Br J Cancer* **12**, 2277-2283.
3. Alsbeih G, Malone S, Lochrin C, *et al.* (2000). Correlation between normal tissue complications and *in vitro* radiosensitivity of skin fibroblasts derived from radiotherapy patients treated for variety of tumors. *Int J Radiat Oncol Biol Phys* **1**, 143-152.
4. Slonina D, Gasinska A (1997). Intrinsic radiosensitivity of healthy donors and cancer patients as determined by the lymphocyte micronucleus assay. *Int J Radiat Biol* **6**, 693-701.
5. Leong T, Borg M, McKay M (2004). Clinical and cellular radiosensitivity in inherited human syndromes. *Clin Oncol (R Coll Radiol)* **3**, 206-209.
6. West C M, Davidson S E, Elyan S A, *et al.* (2001). Lymphocyte radiosensitivity is a significant prognostic factor for morbidity in carcinoma of the cervix. *Int J Radiat Oncol Biol Phys* **1**, 10-15.
7. Barber J B, Burrill W, Spreadborough A R, *et al.* (2000). Relationship between *in vitro* chromosomal radiosensitivity of peripheral blood lymphocytes and the expression of normal tissue damage following radiotherapy for breast cancer. *Radiother Oncol* **2**, 179-186.
8. Smart V, Curwen G B, Whitehouse C A, *et al.* (2003). Chromosomal radiosensitivity: a study of the chromosomal G (2) assay in human blood lymphocytes indicating significant inter-individual variability. *Mutat Res* **1-2**, 105-110.
9. Ban S, Konomi C, Iwakawa M, *et al.* (2004). Radiosensitivity of peripheral blood lymphocytes obtained from patients with cancers of the breast, head and neck or cervix as determined with a micronucleus assay. *J Radiat Res (Tokyo)* **4**, 535-541.

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10. Mariano Ruiz de Almodovar J, Guirado D, Isabel Nunez M, *et al.* (2002). Individualization of radiotherapy in breast cancer patients: possible usefulness of a DNA damage assay to measure normal cell radiosensitivity. *Radiother Oncol* **3**, 327-333.
11. Moneef M A, Sherwood B T, Bowman K J, *et al.* (2003). Measurements using the alkaline comet assay predict bladder cancer cell radiosensitivity. *Br J Cancer* **12**, 2271-2276.
12. Fenech M (2002). Chromosomal biomarkers of genomic instability relevant to cancer. *Drug Discov Today* **22**, 1128-1137.
13. Mozdarani H, Mansouri Z, Haeri S A (2005). Cytogenetic radiosensitivity of g0-lymphocytes of breast and esophageal cancer patients as determined by micronucleus assay. *J Radiat Res (Tokyo)* **1**, 111-116.
14. Jianlin L, Jiliang H, Lifan J, *et al.* (2004). Measuring the genetic damage in cancer patients during radiotherapy with three genetic end-points. *Mutagenesis* **6**, 457-464.
15. Jianhua Z, Lian X, Shuanlai Z, *et al.* (2006). DNA lesion and Hprt mutant frequency in rat lymphocytes and V79 Chinese hamster lung cells exposed to cadmium. *J Occup Health* **2**, 93-99.
16. Strauss G H, Albertini R J (1979). Enumeration of 6-thioguanine-resistant peripheral blood lymphocytes in man as a potential test for somatic cell mutations arising *in vivo*. *Mutat Res* **2**, 353-379.
17. Albertini R J, Anderson D, Douglas G R, *et al.* (2000). IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. *Mutat Res* **2**, 111-172.
18. Yang J L, Chao J I, Lin J G (1996). Reactive oxygen species may participate in the mutagenicity and mutational spectrum of cadmium in Chinese hamster ovary-K1 cells. *Chem Res Toxicol* **8**, 1360-1367.
19. Norman A, Mitchell J C, Iwamoto K S (1988). A sensitive assay for 6-thioguanine-resistant lymphocytes. *Mutat Res* **1**, 17-19.
20. Jones I M, Thomas C B, Haag K, *et al.* (1999). Total gene deletions and mutant frequency of the HPRT gene as indicators of radiation exposure in Chernobyl liquidators. *Mutat Res* **2**, 233-246.
21. Fenech M, Morley A A (1985). Measurement of micronuclei in lymphocytes. *Mutat Res* **1-2**, 29-36.
22. Fenech M (2000). The *in vitro* micronucleus technique. *Mutat Res* **1-2**, 81-95.
23. Chen Z, Lou J, Chen S, *et al.* (2006). Evaluating the genotoxic effects of workers exposed to lead using micronucleus assay, comet assay and TCR gene mutation test. *Toxicology* **3**, 219-226.
24. Zhang M B, He J L, Jin L F, *et al.* (2002). Study of low-intensity 2450-MHz microwave exposure enhancing the genotoxic effects of mitomycin C using micronucleus test and comet assay *in vitro*. *Biomed Environ Sci* **15**, 283-290.
25. Singh N P, McCoy M T, Tice R R, *et al.* (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* **1**, 184-191.
26. Cao J, Liu Y, Sun H, *et al.* (2002). Chromosomal aberrations, DNA strand breaks and gene mutations in nasopharyngeal cancer patients undergoing radiation therapy. *Mutat Res* **1-2**, 85-90.
27. Maluf S W (2004). Monitoring DNA damage following radiation exposure using cytokinesis-block micronucleus method and alkaline single-cell gel electrophoresis. *Clin Chim Acta* **1-2**, 15-24.
28. Riches A C, Bryant P E, Steel C M, *et al.* (2001). Chromosomal radiosensitivity in G2-phase lymphocytes identifies breast cancer patients with distinctive tumour characteristics. *Br J Cancer* **8**, 1157-1161.
29. Hoeller U, Borgmann K, Bonacker M, *et al.* (2003). Individual radiosensitivity measured with lymphocytes may be used to predict the risk of fibrosis after radiotherapy for breast cancer. *Radiother Oncol* **2**, 137-144.
30. Baeyens A, Thierens H, Claes K, *et al.* (2002). Chromosomal radiosensitivity in breast cancer patients with a known or putative genetic predisposition. *Br J Cancer* **12**, 1379-1385.
31. Burrill W, Barber J B, Roberts S A, *et al.* (2000). Heritability of chromosomal radiosensitivity in breast cancer patients: a pilot study with the lymphocyte micronucleus assay. *Int J Radiat Biol* **12**, 1617-1619.
32. Kubota M, Lin Y W, Hamahata K, *et al.* (2000). Cancer chemotherapy and somatic cell mutation. *Mutat Res* **2**, 93-102.
33. Schabath M B, Spitz M R, Grossman H B, *et al.* (2003). Genetic instability in bladder cancer assessed by the comet assay. *J Natl Cancer Inst* **7**, 540-547.

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