Genetics and Molecular Biology, 23, 4, 1101-1105 (2000)

Mini-Review

Predisposition to cancer and radiosensitivity*

P. Pichierri, A. Franchitto and F. Palitti

Università degli Studi della Tuscia, Dipartimento di Agrobiologia ed Agrochimica (DABAC), Via S. Camillo de Lellis, I-01100 Viterbo, Italy. Send correspondence to F.P. Fax: +39-0761-357-242. E-mail: palitti@unitus.it

*Presented at the International Graduate School Course and Workshop on "New Approaches in the Study of Radiation-Induced and Cancer-Associated Chromosomal Aberrations"

Abstract

Many cancer-prone diseases have been shown to be radiosensitive. The radiosensitivity has been attributed to pitfalls in the mechanisms of repair of induced DNA lesions or to an impaired cell cycle checkpoint response. Although discrepancies exist in the results obtained by various authors on the radiosensitivity of individuals affected by the same disease, these can be attributed to the large variability observed already in the response to radiation of normal individuals. To date three test are commonly used to assess radiosensitivity in human cells: survival, micronucleous and G_2 chromosomal assay. The three tests may be performed using either fibroblasts or peripheral blood lymphocytes and all the three tests share large interindividual variability. In this regard a new approach to the G_2 chromosomal assay which takes into account the eventual differences in cell cycle progression among individuals has been developed. This new approach is based on the analysis of G_2 homogeneous cell populations. Cells irradiated are immediately challenged with medium containing bromodeoxyuridine (BrdUrd). Then cells are sampled at different post-irradiation times and BrdUrd incorporation detected on metaphases spread and the scoring is done only at time points showing similar incidence of labelled cells among the different donors. Using this approach it has been possible to reduce the interindividual variability of the G_2 chromosomal assay.

INTRODUCTION: RADIOSENSITIVITY AND HUMAN SYNDROMES

Interest in the radiosensitivity of human cells has developed from the identification of a series of genetic disorders that not only exhibit large, unambiguous alterations in sensitivity to radiation and genotoxic chemicals but are also cancer-prone.

Xeroderma pigmentosum (XP) was the first disease to be associated with a major alteration in radiation response (Cleaver, 1968, 1969). XP patients exhibit a hereditary susceptibility to sunlight-induced cancer correlated with a genetic defect in the ability to repair DNA damage (Cleaver and Kraemer, 1989). Fibroblasts and lymphoid cells from XP patients showed hypersensitivity to ultraviolet (UV) light damage *in vitro*. In this view, many investigations of the relationship between radiation sensitivity and cancer susceptibility in a wide range of genetic disorders were performed, and it was demonstrated that several other diseases are also characterized by a hypersusceptibility to DNA-damaging agents, like Fanconi's anemia syndrome, sensitive to DNA cross-linking agents (Fujiwara and Tatsumi, 1977) and Werner's syndrome, susceptible to the carcinogen 4-nitroquinoline-oxide (Gebhart *et al*., 1985).

A deficiency in DNA repair was clearly demonstrated to be an underlying cause of UV-sensitivity in XP and Cockayne syndrome, but more complex abnormalities in the processing of DNA damage induced by X-irradiation were identified in ataxia telangiectasia (AT).

Evidence from these disorders sets the stage for the idea that a wide range of hereditary clinical disorders could be related in varying degrees to an increased sensitivity to DNA-damaging agents and radiosensitivity.

The most striking example is AT, an autosomal recessive disorder that causes immunological dysfunction, proneness to cancer, and an unusual susceptibility to X-irradiation (Higurashi and Conen, 1973; for a recent review see Lavin and Khanna, 1999). Also the Nijmegen syndrome, an autosomal recessive disease previously identified as AT variant, is characterized by a high sensitivity to X-irradiation (see Digweed *et al*., 1999). It has also been demonstrated that many other syndromes or inherited conditions are characterized by increased radiosensitivity (Table I). Among them, both hereditary syndromes characterized by wide genomic instability (e.g., Bloom's syndrome) and cancer syndromes (Wilm's tumor).

It is noteworthy that almost all these pathological situations are reported to indicate predisposition towards cancer in the affected individuals. For this reason, it could be very interesting to assess whether a strong correlation between radiosensitivity and predisposition to cancer exists.

A typical example are AT heterozygotes which are more radiosensitive than healthy controls and have an ap-

Table I - Syndromes exhibiting radiosensitivity.

Pathological condition	References*
Ataxia telangiectasia homozygotes	Higurashi and Conen (1973); Sanford *et al.* (1990)
Ataxia telangiectasia heterozygotes	Sanford *et al.* (1990); Scott *et al.* (1994)
Basal cell nevus syndrome	Featherstone *et al.* (1983)
Bloom's syndrome	Higurashi and Conen (1973); Kuhn (1980)
Common variable immune disorder	Vorechovsky *et al.* (1993)
Down's syndrome	Sasaki *et al.* (1970); Morten *et al.* (1991)
Dyskeratosis congenita	DeBauche *et al.* (1990)
Epidermodysplasia verruciformis	El-Zein *et al.* (1995)
Familial dysplastic nevus syndrome	Sanford *et al.* (1987)
Fanconi's anemia	Higurashi and Conen (1973); Parshad *et al.* (1983); Duckworth-Rysiecki and Taylor (1985)
Gardner's syndrome	Parshad *et al.* (1983)
Klinefelter syndrome	Sasaki *et al.* (1970)
Li-Fraumeni syndrome	Parshad *et al.* (1993)
Nijmegen breakage syndrome	Talman *et al.* (1983); Jaspers *et al.* (1988)
Rothmund Thomson syndrome	Kerr *et al.* (1996)
Retinoblastoma (familial)	Morten *et al.* (1991); Sanford *et al.* (1996)
Wilm's tumour	Sanford *et al.* (1989)
Xeroderma pigmentosum	Price *et al.* (1991)

*For bibliographic references see: Scott *et al.* 1996.

proximately 4-fold increased risk of breast cancer (Easton, 1994). Radiosensitivity has been extensively studied in breast cancer patients (Samouhos, 1983; Rigaud *et al.*, 1990; Scott *et al.*, 1998; Roberts *et al.*, 1999; Barber *et al.*, 2000). To date mutations in two genes, BRCA1 and BRCA2, have been found to correlated to breast cancer predisposition. However, only 5% of breast cancer patients show mutations in either BRCA1 or BRCA2 genes (Goldgar *et al.*, 1996; Ford and Easton, 1996).

In contrast, about 40% of an unselected group of breast cancer cases were found to be radiosensitive (Scott *et al.*, 1994). This finding suggests that radiosensitivity could actually be a potential predisposing condition to breast cancer (Roberts *et al.*, 1999). In the study of Scott and colleagues (1994), the authors also found that about 9% of healthy controls were radiosensitive.

Many other studies were performed to determine the incidence of radiosensitive individuals in the general population. For example, Sanford and colleagues (1989) reported that about 5% of the normal population is radiosensitive.

However, discrepancies exist in the results presented in each study. In particular, conditions proved to be radiosensitive by one author are not by others (Scott *et al.*, 1996; Palitti *et al.*, 1999). One of the recognized problems is the variability in the response to radiation of apparently normal individuals. This problem was extensively investigated by Little and colleagues (1989), who demonstrated that great variability exists in the response to radiation of normal cells. This finding complicates the use of radiosensitivity as a marker of cancer predisposition. In fact, this variability makes it very difficult to determine the range in which the individuals are to be considered radiosensitive or normal. Moreover, it is not proven that these are always cancer-prone conditions and thus all the individuals predisposed to cancer have to be radiosensitive. In fact, radiosensitivity would result, from defects in the repair of radiation-induced DNA damage or defects in the processing and/or signalling of this damage.

In this regard, a lot of genes could be involved in the radiosensitive phenotype, only a few of which are known (Table II) and it is unlikely that all these genes can be correlated with predisposition to cancer.

On the other hand, apparently normal radiosensitive individuals could be examined to discover new genes implicated in the radiation response.

PATHWAYS INVOLVED IN THE REPAIR OF IONIZING RADIATION-INDUCED DOUBLE-STRAND BREAKS

Double-strand breaks (DSB) are generated by endogenously produced radicals and exogenous agents such as ionizing radiation (IR), which is often used in anti-cancer therapy. Repair of DSBs is of cardinal importance to prevent chromosomal fragmentation, translocations and deletions. In the soma, the genomic instability resulting from persistent or incorrectly repaired DSBs can lead to carcinogenesis through activation of oncogenes, inactivation of tumor-suppressor genes or loss of heterozygosity, while in the germline they can lead to inborn defects (see Kanaar *et al.*, 1998).

The deleterious effects of DSBs have triggered the evolution of multiple pathways for their repair (see Kanaar *et al.*, 1998; Critchlow and Jackson, 1998; Pastink and Lohman, 1999).

i) single-strand annealing (SSA),

ii) non-homologous end-joining (NHEJ),

iii) recombinational repair (RR) see Figure 1.

All three mechanisms are conserved in evolution but their relative contributions differ between higher and lower eukaryotes. The relative importance of each pathway also depends on the phase of the cell cycle, NHEJ being mostly active during G1/early-S but RR and SSA active only during late-S/G2 (Takata *et al.*, 1998).

Homologous recombination requires extensive regions of DNA homology and repairs DSBs accurately using information on the undamaged sister chromatid or homologous chromosome. This pathway depends upon the presence of the protein products of the RAD52 epistasis group of genes. These proteins, which appear conserved from yeast to man, essentially act as during meiotic crossing-over (see Figure 1; Haber, 2000).

DNA end-joining, on the other hand, uses no, or ex-

Table II - Genes involved in the repair and processing of IR-induced DNA damage.

Gene	Disease	Cellular phenotype
ATM	Ataxia telangiectasia	IR-sensitive, checkpoint defects, impaired DNA damage signalling
MRE11	Ataxia telangiectasia-like disorder	IR-sensitive, checkpoint defects
NBS1	Nijmegen-breakage syndrome	IR-sensitive, partial checkpoint defects, impaired DNA damage signalling
RAD50	Unknown	Very IR-sensitive. Embryonic lethal (mouse)
KU70	SCID	IR-sensitive, reduced end-joining, DSB repair defective
KU80	SCID	IR-sensitive, reduced end-joining, DSB repair defective, chromosomal rearrangements (mouse)
DNA-PKcs	SCID	IR-sensitive, slightly reduced end-joining, DSB repair defective, defect in the recovery from G2 arrest
LIG4	Unknown, possible predisposition to leukemia	IR-sensitive, end-joining absent, large chromosomal rearrangements (mouse)
XRCC4	Unknown	Unknown
RAD51	Not applicable	Embryonic lethal. Very IR-sensitive, G2-phase checkpoint defective, large chromosomal rearrangements in conditioned nullozygous chicken DT40 cells
RAD52	Unknown	IR-sensitive, slightly impaired DSB repair, chromosomal rearrangements (mouse)
RAD51B-D	Unknown	Unknown
XRCC2	Unknown	Slightly IR-sensitive, proficient DSB repair
XRCC3		DNA-crosslink sensitive

tremely limited, sequence homology to rejoin juxtaposed ends in a manner that need not be error free (Figure 1). This repair system, first identified in mammals, has also been found in yeast; it repairs predominantly through RR, and it is dependent upon the activity of the DNA-PK complex and that of XRCC4/Ligase 4 (Jeggo *et al*., 1999).

The SSA pathway was proposed initially to explain the results of intramolecular recombination in plasmid DNAs introduced into mammalian cells or *X. laevis* oocytes. Later on it became evident that repair by SSA also occurs in yeast. The SSA pathway is dependent on the presence of direct repeats on both sides of the break and thus does not require extensive homology. This pathway depends upon the presence of the RAD52 protein as well as of the mismatch repair enzyme MSH2 and MSH3, at least in yeast (Pastink and Lohman, 1999).

Not only are the enzymes of the DSB repair systems important for determining the cellular sensitivity to ionizing radiation but also those involved in the signalling of the DNA damage to the checkpoint mechanism. This is proved by the radiosensitive phenotype of the human syndrome ataxia telangiectasia, in which large defects in the checkpoint activation are present. (See Table II for a summary of the properties of the proteins involved in the response to IR-induced DSBs and the corresponding cellular phenotypes in mammalian cells).

The common use of IR in anti-tumor radiotherapy, as well as the observation of links between radiosensitivity and cancer proneness, make it paramount to give attention into the molecular intricacies of IR-induced DNA damage repair when looking for a radiosensitive phenotype. In the past few years, the availability of known genes has provided the essential tools to translate the results from the initial genetic analyses to molecular mechanisms of DSB repair. Currently, a major challenge ahead is the evaluation of the relative contribution of the different DSB repair pathways survival after exposure to IR in different mammalian tissues and tumors in the view of assessing the risks arising from radiation exposure.

RADIOSENSITIVITY ASSAYS

To date, three tests are commonly used to assess radiosensitivity in human cells:

- ♦ Survival assay;
- ♦ Micronucleus assay;
- ♦ G2 chromosomal assay.

The first uses the survival after irradiation with different doses to obtain and compare the D0 values (D0 values are the dose value at which the survival is reduced to 37% of the untreated culture). A low D0 value should correspond to a radiosensitive phenotype.

The second looks at the X-ray-induced micronuclei, both after low and high irradiation rates (giving better reproducible results according to Scott *et al*., 1998). Higher yields of micronuclei correlate with radiosensitivity.

The third uses the induction of chromatid-type aberrations after irradiation (50 cGy usually) in the G2-phase of the cell cycle. Also in this case, higher yields of chromosomal damage correlate with radiosensitivity.

The three tests may be performed using either fibroblasts or peripheral blood lymphocytes, the former being preferred for the survival assay. All three tests share the interindividual variability described above. Reasons for this variability have been attributed to differences in the experimental procedures used by different investigators (Scott *et al*., 1996) or to differences in cellular growth among the individuals (Darroudi *et al*., 1995; Palitti *et al*., 1999).

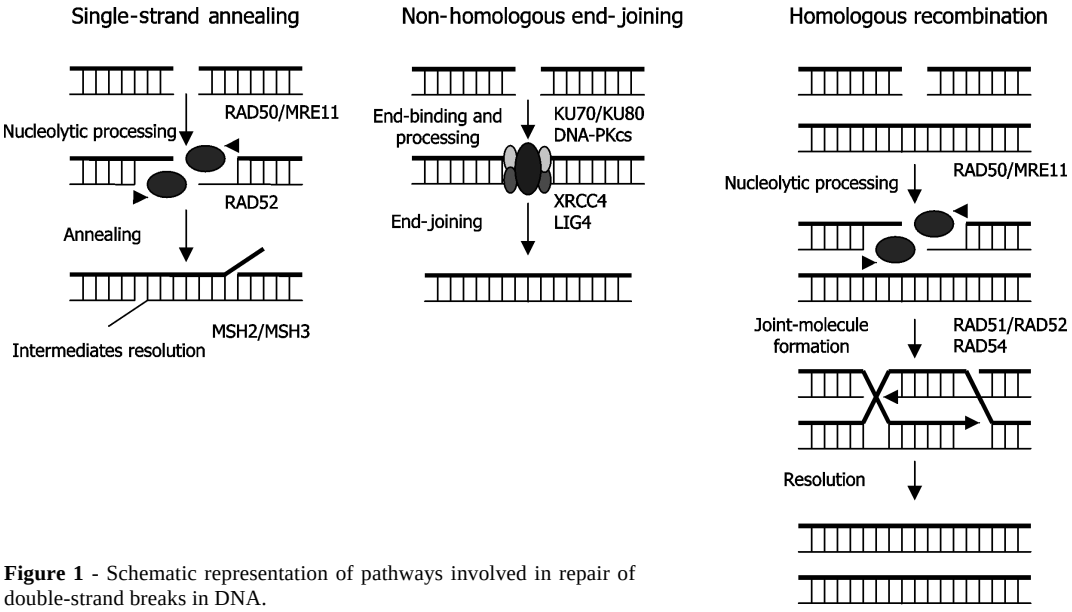

Figure 1 - Schematic representation of pathways involved in repair of double-strand breaks in DNA.

For example, in the survival assay, differences in the relative duration of G1, S and G2 phases of the cell cycle, which are not uniform in their sensitivities could influence radiation sensitivity. On the other hand, different responses in the duration of the G2 arrest after irradiation could give a large variability in the yield of chromosomal damage using the G2 assay.

In this regard, a new approach to the G2 assay, which takes into account these differences in the extent of the X-ray-induced G2 delay has been developed by Palitti and colleagues (1999). This new approach is based on the analysis of the G2 homogeneous population in mitosis. Cells are irradiated and immediately challenged in medium containing bromodeoxyuridine (BrdUrd). Then, cells are harvested at different post-irradiation times and BrdUrd incorporation is visualized by means of immunocytochemical detection on metaphase spreads. For each time-point the percentage of labelled cells in mitosis is calculated and chromosomal damage scored in unlabelled mitosis (G2) only at time-points showing a similar incidence of labelled cells (usually around 10%). Using this approach, the authors have actually been able to reduce the interindividual variability of the G2 assay.

CONCLUDING REMARKS

It may be useful to develop a simple cytogenetic assay which would allow the screening of a large number of individuals for radiosensitivity in order to direct the positive cases to more relevant screening for cancer susceptibility. In addition, unambiguous identification of radiosensitive patients with cancer could be of importance for the correct use of the radiotherapy in oncology. Both situations require a good test for radiosensitivity.

RESUMO

Muitas doenças que predispõem ao câncer têm se mostrado radiossensíveis. A radiossensibilidade tem sido atribuída a problemas nos mecanismos de reparo de lesões de DNA induzidas ou a uma resposta alterada no "checkpoint" do ciclo celular. Embora existam discrepâncias entre os resultados obtidos por vários autores quanto à radiossensibilidade de indivíduos afetados pela mesma doença, essas discrepâncias podem ser atribuídas à grande variabilidade observada já na resposta de indivíduos normais à radiação. Até hoje, três testes têm sido comumente usados para avaliar a radiossensibilidade em células humanas: sobrevivência, micronúcleo e ensaio cromossômico em G2. Os três testes podem ser realizados usando tanto fibroblastos como linfócitos do sangue periférico e todos os três testes têm em comum grande variabilidade interindividual. Uma nova abordagem ao ensaio cromossômico em G2 que leva em consideração as eventuais diferenças entre indivíduos quanto à progressão do ciclo celular foi desenvolvida. Esta nova abordagem é baseada na análise de populações celulares em G2 homogêneas. Células são irradiadas e imediatamente estimuladas com meio contendo bromodeoxiuridina (BrdUrd). Então as células são amostradas em diferentes tempos pós-irradiação e a incorporação de BrdUrd é detectada em esfregaços de metáfases, sendo a contagem feita apenas em períodos de tempo que mostrem incidência similar de células marcadas em diferentes doadores. Usando essa abordagem foi possível reduzir a variabilidade interindividual do ensaio cromossômico em G2.

REFERENCES

Barber, J.B., Burrill, W., Spreadborough, A.R., Levine, E., Warren, C., Kiltie, A.E., Roberts, S.A. and **Scott, D.** (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. 55*: 179-186.

Cleaver, J.E. (1968). Defective repair replication of DNA in xeroderma

pigmentosum. *Nature 218*: 652-656.

Cleaver, J.E. (1969). Xeroderma pigmentosum: a human disease in which an initial stage of DNA repair is defective. *Proc. Natl. Acad. Sci. USA 63*: 428-435.

Cleaver, J.E. and **Kraemer, K.H.** (1989). Xeroderma pigmentosum. In: *The Metabolic Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D., eds.). 6th edn., Vol. II. McGraw-Hill, New York, pp. 2946-2971.

Critchlow, S.E. and **Jackson, S.P.** (1998). DNA end-joining: from yeast to man. *Trends Biochem. Sci. 23*: 394-398s.

Darroudi, F., Vyas, R.C., Vermeulen, S. and **Natarajan, A.T.** (1995). G2 radiosensitivity of cells derived from cancer-prone individuals. *Mutat. Res. 328*: 83-90.

Digweed, M., Reis, A. and **Sperling, K.** (1999). Nijmegen breakage syndrome: consequences of defective DNA double strand break repair. *Bioessays 21*: 649-656.

Easton, D.F. (1994). Cancer risks in A-T heterozygotes. *Int. J. Radiat. Biol. 66*: S177-S182.

Ford, D. and **Easton, D.F.** (1996). The breast-ovarian cancer syndrome and BRCA1. In: *Genetic Predisposition to Cancer* (Eeles, R.A., Ponder, B.A.J., Easton, D.F. and Horwhich, A., eds.). Chapman and Hall, London, pp. 239-252.

Fujiwara, Y. and **Tatsumi, M.** (1977). Cross-link repair in human cells and its possible defect in Fanconi's anemia cells. *J. Mol. Biol. 113*: 635-649.

Gebhart, E., Schinzel, M. and **Ruprecht, K.W.** (1985). Cytogenetic studies using various clastogens in two patients with Werner syndrome and control individuals. *Hum. Genet. 70*: 324-327.

Goldgar, D.E., Strattonn, M.R. and **Eles, R.A.** (1996). Familial breast cancer. In: *Genetic Predisposition to Cancer* (Eeles, R.A., Ponder, B.A.J., Easton, D.F. and Horwhich, A., eds.). Chapman and Hall, London, pp. 227-238.

Haber, J.E. (2000). Recombination: a frank view of exchanges and vice versa. *Curr. Opin. Cell Biol. 12*: 286-296.

Higurashi, M. and **Conen, P.E.** (1973). *In vitro* chromosomal radiosensitivity in "chromosomal breakage syndromes". *Cancer 32*: 380-383.

Jeggo, P., Singleton, B., Beamish, H. and **Priestley, A.** (1999). Double strand break rejoining by the Ku-dependent mechanism of non-homologous end-joining. *C.R. Acad. Sci. III 322*: 109-112.

Kanaar, R., Hoeijmakers, J.H. and **van Gent, D.C.** (1998). Molecular mechanisms of DNA double strand break repair. *Trends Cell. Biol. 8*: 483-489.

Lavin, M.F. and **Khanna, K.K.** (1999). ATM: the protein encoded by the gene mutated in the radiosensitive syndrome ataxia-telangiectasia. *Int. J. Radiat. Biol. 75*: 1201-1214.

Little, J.B., Nichols, W.W., Troilo, P., Nagasawa, H. and **Strong, L.C.** (1989). Radiation sensitivity of cell strains from families with genetic disorders predisposing to radiation-induced cancer. *Cancer Res. 49*: 4705-4714.

Palitti, F., Pichierri, P., Franchitto, A., Proietti De Santis, L. and **Mosesso, P.** (1999). Chromosome radiosensitivity in human G2 lymphocytes and cell-cycle progression. *Int. J. Radiat. Biol. 75*: 621-627.

Pastink, A. and **Lohman, P.H.** (1999). Repair and consequences of double-strand breaks in DNA. *Mutat. Res. 428*: 141-156.

Rigaud, O., Guedeney, G., Duranton, I., Leroy, A., Doloy, M.T. and **Magdelenat, H.** (1990). Genotoxic effects of radiotherapy and chemotherapy on the circulating lymphocytes of breast cancer patients. II. Alteration of DNA repair and chromosome radiosensitivity. *Mutat. Res. 242*: 25-35.

Roberts, S.A., Spreadborough, A.R., Bulman, B., Barber, J.B., Evans, D.G. and **Scott, D.** (1999). Heritability of cellular radiosensitivity: a marker of low-penetrance predisposition genes in breast cancer? *Am. J. Hum. Genet. 65*: 784-794.

Samouhos, E. (1983). Chromosomes, cancer and radiosensitivity. *Am. J. Clin. Oncol. 6*: 503-506.

Sanford, K.K., Parshad, R., Gantt, R., Tarone, R.E., Jones, G.M. and **Price, F.M.** (1989). Factors affecting and significance of G2 chromatin radiosensitivity in predisposition to cancer. *Int. J. Radiat. Biol. 55*: 963-981.

Scott, D., Spreadborough, A., Levine, E. and **Roberts, S.A.** (1994). Genetic predisposition in breast cancer. *Lancet 344*: 1444.

Scott, D., Spreadborough, A.R., Jones, L.A., Roberts, S.A. and **Moore, C.J.** (1996). Chromosomal radiosensitivity in G2-phase lymphocytes as an indicator of cancer predisposition. *Radiat. Res. 145*: 3-16.

Scott, D., Barber, J.B., Levine, E.L., Burrill, W. and **Roberts, S.A.** (1998). Radiation-induced micronucleus induction in lymphocytes identifies a high frequency of radiosensitive cases among breast cancer patients: a test for predisposition? *Br. J. Cancer 77*: 614-620.

Takata, M., Sasaki, M.S., Sonoda, E., Morrison, C., Hashimoto, M., Utsumi, H., Yamaguchi-Iwai, Y., Shinohara, A. and **Takeda, S.** (1998). Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity of vertebrate cells. *EMBO J.: 17*: 5497-5508.

(Received November 23, 2000)