Irradiation-mediated Rescue of T Cell–specific V(D)J Recombination and Thymocyte Differentiation in Severe Combined Immunodeficient Mice by Bone Marrow Cells

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Summary

In SCID (severe combined immunodeficient) mice, proper assembly of immunoglobulin and T cell receptor (TCR) genes is blocked by defective V(D)J recombination so that B and T lymphocyte differentiation is arrested at an early precursor stage. Treating the mice with gamma irradiation rescues V(D)J rearrangement at multiple TCR loci, promotes limited thymocyte differentiation, and induces thymic lymphomas. These effects are not observed in the B cell lineage. Current models postulate that irradiation affects intrathymic T cell precursors. Surprisingly, we found that transfer of irradiated SCID bone marrow cells to unirradiated host animals rescues both TCR rearrangements and thymocyte differentiation. These data indicate that irradiation affects precursor cells at an earlier stage of differentiation than was previously thought and suggest new models for the mechanism of irradiation rescue.

Key words: T cell receptors • lymphocyte differentiation • hematopoietic stem cells • TCR rearrangement • adoptive transfer

The adaptive immune response provides vertebrate organisms with potent defenses against invading pathogens. Antigen receptor diversity is critical to the success of this defense system. TCR and Ig molecules are required for immune effector functions that are initiated by recognition of foreign antigens. These receptors, expressed on the surfaces of developing T and B cells, are also required for lymphocyte differentiation.

Receptor diversity is achieved through a site-specific DNA rearrangement mechanism, V(D)J recombination, which is responsible for assembling the variable (V), diversity (D), and joining (J) regions of Ig and TCRs during lymphocyte differentiation. The reaction can be divided into two steps: DNA cleavage and joining of the broken ends. Cleavage, mediated by the recombination-activating gene products RAG-1 and RAG-2, produces two kinds of DNA termini, signal ends and coding ends. These intermediates join to form signal and coding joints, respectively.

Rearrangement of the TCR genes is intimately connected to thymocyte differentiation, which begins with the commitment of bone marrow hematopoietic stem cells to the T cell lineage (for review see reference 1). The earliest T lineage precursors in the thymus have TCR genes in the germline state (1–3). Differentiation of the α/β T cell lineage can be conveniently followed by monitoring cell surface expression of CD3 (a noncovalently associated complex involved in TCR signaling) and the CD4 and CD8 coreceptor molecules. Early intrathymic precursors are CD4–CD8– double negative (DN)1 cells that typically express CD3 at very low levels. TCR-β rearrangement begins at the DN stage. Expression of a functional TCR-β chain allows thymocytes to mature to the next stage, in which both CD4 and CD8, as well as low to intermediate levels of CD3, are expressed. TCR-α rearrangements occur in these double positive (DP) thymocytes. After productive TCR-α rearrangement and selection, thymocytes become mature single positive (SP) cells, expressing high levels of CD3 and either CD4 or CD8 at the cell surface.

Because expression of functional TCR chains is required for thymocyte differentiation, thymocytes of mice bearing genetic lesions that block V(D)J recombination, such as RAG-deficient and SCID mice, are arrested at the DN stage. RAG-deficient mice are unable to initiate V(D)J recombination because the RAG proteins are required for site-specific DNA cleavage. In contrast, SCID mice, which are defective for the catalytic subunit of the DNA-dependent protein kinase, initiate recombination normally but are defective in the joining of coding ends, which leads to accumulation of these recombination intermediates (4).

Treatment of adult or newborn SCID mice with low doses of DNA damaging agents such as gamma irradiation rescues V(D)J recombination at multiple TCR loci, including β, γ, and δ, and promotes thymocyte proliferation and

1Abbreviations used in this paper: DN, double negative; DP, double positive; SP, single positive.
differentiation to the DP stage (5–9). Curiously, the effects of irradiation appear to be restricted to the T cell lineage, as neither rescue of Ig rearrangements nor B cell differentiation is observed in irradiated SCID mice (5, 6). Radiation also exerts a profound oncogenic effect on these animals, as all treated SCID mice develop thymic lymphomas within a few months of irradiation (5, 6), compared with a low incidence of thymic lymphoma in untreated SCID mice.

Because the irradiation effects were observed in thymocytes but not B cell precursors, it has been thought that the cells responsible for the effect reside in the thymus at the time of irradiation (5–9). The prevailing view is that irradiation facilitates a transient “bypass” of the SCID defect, promoting the joining of preexisting hairpin coding ends (5–9). This is consistent with the observation that rearrangements are rescued at loci that are actively undergoing recombination at the time of irradiation (TCR-β, -δ, and -γ) but not at the TCR-α locus (7–9), which is recombinationally silent in DN thymocytes (7, 8). The timing of the appearance of DP thymocytes and rearrangements after irradiation is consistent with the kinetics of repopulation of irradiated wild-type thymi from intrathymic, radioresistant thymocyte precursors (10, 11). Therefore, we proposed that radioresistant DN thymocytes may be the targets for the irradiation effects (8).

Although the data described above are consistent with the hypothesis that rescue of thymocyte differentiation and V(D)J recombination is mediated by effects on intrathymic precursor cells, the cellular targets of irradiation have not been identified. In addition to thymocytes, potential targets include bone marrow cells (T lineage precursors or other elements), as well as thymic stromal cells, which might play crucial roles in the irradiation response by altering the expression of cell surface molecules or releasing cytokines capable of promoting thymocyte differentiation (12–14). Indeed, transfer of mature T cells or wild-type bone marrow to SCID mice can promote differentiation of SCID thymocytes, presumably because of inductive effects on the thymic microenvironment (15, 16).

To address these issues, we employed an adoptive transfer approach. Our data show that transfer of irradiated adult SCID bone marrow rescues both TCR rearrangements and thymocyte differentiation. Thus, exposure of thymocytes or thymic stromal cells to ionizing radiation is not required for the irradiation rescue effect. These results indicate that, surprisingly, the cellular targets of irradiation include early lymphocyte progenitor cells residing in the bone marrow and lead us to consider new models for the mechanism of irradiation rescue.

Materials and Methods

Mice and Irradiation. C.B.17 scid/scid and 129 × C57BL pfp/rag-2 double-knockout (RAG/NK-) mice (Taconic) were maintained in microisolator cages in our animal facility at Baylor College of Medicine. Age-matched BALB/c mice were used as controls. Mice were irradiated (2 Gy) by exposure to a Cs source.

A adoptive transfer. Intrathymic injections were performed as described (17). In brief, mice were anesthetized by methoxyflu-
not shown). These data could indicate that intrathymic precursors are not the major mediators of the irradiation rescue effect; however, we cannot rule out the possibility that technical factors limited our ability to detect rescue.

We next asked whether cellular elements residing in the bone marrow, such as primitive lymphocyte precursors, might be capable of mediating the irradiation response. As a control, we transferred unirradiated wild-type (BALB/c) bone marrow cells (of the same MHC haplotype as SCID mice) by intrathymic injection and analyzed recipient thymi after 16–23 d. RAG/NK \(^{-/-}\) host thymi were reconstituted by injections of wild-type bone marrow, giving near normal to normal cellularity with appreciable proportions of both DP and SP thymocytes (Fig. 1 A; Table I). This effect was not observed after transfer of unirradiated SCID bone marrow cells (Fig. 1 B). In four out of five experiments, intrathymic injection of adult SCID bone marrow cells harvested immediately after whole body irradiation resulted in increased thymic cellularity and appearance of a distinct population of DP thymocytes in host animals after 16–23 d (Fig. 1 C; Table I). Representative unirradiated and irradiated SCID thymocyte profiles are shown for comparison (Fig. 1 D and E). In three out of five animals, distinct populations of CD4 SP cells (4–7% of total thymocytes) were also apparent. Further analysis of this CD4\(^{+}\) population revealed high levels of surface CD3 (Fig. 1 C) similar to those in the wild-type controls (Fig. 1 A). Consistent with this finding, CD3 levels on the surfaces of the DP cells arising in transfers from irradiated SCID bone marrow were higher than those seen in irradiated SCID thymocytes (Fig. 1 C). These data suggest that the transfer of irradiated bone marrow to RAG/NK \(^{-/-}\) hosts results in even more effective rescue of thymocyte differentiation than simple irradiation of SCID mice. However, it should be noted that populations of SP thymocytes and stimulation of intermediate levels of CD3 expression have been observed previously in irradiated adult (6) and newborn (5) SCID mice.

To determine whether V(D)J recombination was rescued in RAG/NK \(^{-/-}\) hosts reconstituted with irradiated SCID bone marrow cells, we employed semiquantitative PCR assays for detection of TCR-\(\beta\), -\(\gamma\), and -\(\alpha\) rearrangements. As expected, TCR-\(\beta\), -\(\gamma\), and -\(\alpha\) coding joints were detected in RAG/NK \(^{-/-}\) hosts reconstituted with wild-type bone marrow cells (Fig. 2, A–C, lane 9). TCR-\(\beta\) and -\(\gamma\) coding joints were detected in thymocytes of RAG/NK \(^{-/-}\) hosts injected with irradiated SCID bone marrow (Fig. 2, A and B, lanes 10 and 11) at levels comparable to those seen in irradiated SCID thymocytes (Fig. 2, A and B, lanes 7 and 8). Sequence analysis of cloned TCR-\(\beta\) rearrangements isolated from irradiated SCID mouse thymocytes revealed that 7/15 junctions recovered from a single animal were unique, and all showed features of TCR-\(\beta\)8-J\(\beta\)2 rearrangements isolated from irradiated SCID mice (5). Only trace amounts of TCR-\(\alpha\) coding joints were detected in recipients of irradiated SCID bone marrow (Fig. 2 C, lanes 10 and 11) or in irradiated SCID thymocytes (Fig. 2 C, lanes 7 and 8), consistent with previous analyses of TCR-\(\alpha\) rearrangements in irradiated SCID mice (8, 9).

![Figure 1.](image)

Figure 1. Transfer of irradiated SCID bone marrow cells rescues CD4\(^{+}\) CD8\(^{-}\) DP cells. CD4, CD8, and CD3 thymocyte profiles are shown from a RAG/NK \(^{-/-}\) host reconstituted with wild-type bone marrow cells (A), a RAG/NK \(^{-/-}\) host reconstituted with wild-type bone marrow cells (B), a RAG/NK \(^{-/-}\) host reconstituted with irradiated SCID bone marrow cells (C), age-matched SCID cells (D), and irradiated (2 Gy) adult SCID cells (E). The first three samples were all harvested 21 d after injection. The numbers in the second quadrants are the percentage of DP cells, the numbers below the histograms denote the cellularity of the thymus, and the numbers in the right panels are the mean fluorescence intensities. In each panel, DP cells were gated for the analysis of the CD3 expression, as shown in the right side one-dimensional histogram. In A and C, CD4\(^{+}\) cells were gated for analysis of CD3 expression.
Table I. Summary of Bone Marrow Transfer into RAG/NK-/- Hosts

| Donor          | N. o. experiments | DP% (cellularity)* |
|---------------|------------------|--------------------|
| IR SCID       | 5                | 74 (19)‡           |
|               |                  | 59 (17)‡           |
|               |                  | 46 (7)             |
|               |                  | 8 (3)              |
|               |                  | 0.4 (3)            |
| Wild-type     | 7                | 84 (140)           |
|               |                  | 85 (71)            |
|               |                  | 84 (50)            |
|               |                  | 74 (170)           |
|               |                  | 67 (74)            |
|               |                  | 49 (33)            |
|               |                  | 32 (20)            |

*10⁶ cells

‡Thymocyte DNA was used for PCR analysis to amplify DNA rearrangements shown in Fig. 2. RAG/NK-/- hosts reconstituted with wild-type bone marrow cells were harvested after 16-21 and 21-23 d; no differences were observed. RAG/NK-/- hosts reconstituted with irradiated (IR) scid bone marrow cells were all harvested after 21-23 d.

Taken together, these results show that irradiated SCID bone marrow cells can promote rescue of both thymocyte differentiation and TCR rearrangements when transferred to unirradiated host animals. These data suggest the possibility that effects on the bone marrow may play an important role in the thymocyte responses observed in irradiated SCID mice.

Discussion

The three cardinal effects of irradiation treatment on differentiation of SCID thymocytes are progression to the DP stage, proliferation, and rescue of V(D)J rearrangements. As these effects are limited to thymocytes, we and others have suggested that these phenomena result from effects of irradiation on intrathymic precursor cells, perhaps aided by important contributions from irradiated thymic stromal cells (5–9). Our data demonstrate that transfer of irradiated bone marrow cells reconstituted all three features. These results show that irradiation of thymic stromal elements is not required for irradiation rescue and indicate that the irradiation effect can be mediated by lymphoid precursor cells at an earlier stage of differentiation than was previously thought.

Can Intrathymic Precursor Cells Mediate Rescue? The failure of irradiated SCID thymocytes to promote rescue of rearrangements or differentiation when transferred to unirradiated host animals could mean that thymocytes are not the targets of the irradiation effect. However, technical factors could have prevented us from detecting an irradiation rescue response in this system. This interpretation is supported by studies of fetal thymic organ cultures. Although rescue of TCR rearrangements was not assessed, differentiation to the DP stage was observed in day 17 fetal thymus placed in organ culture immediately after irradiation of the SCID fetuses (Williams, C., J. Danska, and C. Guidos, personal communication).

Irradiation Rescue Mediated by Bone Marrow Precursors: Mechanistic Implications. The current model for explaining the irradiation rescue effect postulates that irradiation transiently affects double strand break repair pathways in DN thymocytes that are (a) committed to the T cell lineage and (b) actively undergoing rearrangement at the TCR -γ, -δ, and -β loci (5–9). Both of these postulates are undermined by our results, which show that irradiation of SCID mice affects lymphocyte progenitors before they leave the bone marrow, a much earlier stage of T cell differentiation than was previously thought. In fact, lymphocyte precursors capable of repopulating the thymus isolated either from bone marrow or indeed from the thymus are not T lineage restricted and can repopulate the B cell compartment if allowed to enter the bone marrow of a lethally irradiated host (23–25). Although the presence of TCR -β and -γ germ-line transcripts in bone marrow cells (26, 27) suggests that these loci may be accessible to the recombinase machinery, only incomplete (D-J) TCR -β rearrangements have been detected in bone marrow cells by PCR (27). Furthermore, we could not detect V(D)J rearrangements in SCID bone marrow (with or without irradiation) using PCR assays (Wang, C., M. Bogue, and D. Roth, unpublished data). Analysis of DNA from highly purified T lineage precursors.
isolated from murine bone marrow also failed to reveal evidence for TCR-γ or -β rearrangements (1–3). Finally, as the bone marrow lymphoid precursors are not restricted to the T lymphocyte lineage, these cells should not be committed to rearrangement of their TCR loci at this stage.

A New Model for Irradiation-Mediated Rescue of TCR Rearrangements. The considerations described above indicate that irradiation of cells before the onset of V(D)J recombination can rescue rearrangements. This possibility led us to consider a new model for the irradiation rescue effect. We suggest that irradiation induces a persistent response in bone marrow lymphoid progenitor cells that facilitates the joining of V(D)J recombination intermediates created at a later time during lymphocyte differentiation. There are several examples of such an effect in the literature. Increased radioreistance after exposure to low doses of X irradiation has been observed in mammalian cell lines, mouse tissues, and human lymphocytes (for review see reference 28). Based on these and other observations, it has been suggested that low doses (as little as 0.05–1 Gy) of irradiation induce persistent DNA repair activities (for review see reference 28). Induction of these activities in SCID cells might allow coding ends to bypass the SCID defect via an alternative joining mechanism.

These activities would have to persist until the bone marrow precursor cells commit to the T lineage and initiate TCR rearrangements. The length of time required for these events is not known, nor do we know how long radiation-induced radioreistance might persist in this system. In cycling fibroblasts, induced radioreistance can persist for three generations (29). Furthermore, other studies have shown that exposure of cells (including lymphocytes) to a single, low dose of ionizing radiation produces genomic instability that continues for many generations (30, 31). This phenomenon is correlated with an increased incidence of neoplastic transformation (31) and could reflect induction of aberrant recombination or repair activities (30). These observations raise the possibility that radiation-induced alterations in DNA repair activities in bone marrow precursor cells might persist for many cell divisions. Irradiation-induced genomic instability could also be involved in generating the lymphomas that inevitably develop in irradiated SCID mice. It is, therefore, of great interest to determine if irradiated bone marrow precursors are capable of transferring lymphoma to unirradiated host animals.

Unanswered Questions. Why are TCR-α rearrangements not efficiently rescued? We have considered two possible explanations. First, as TCR-α recombines later in differentiation than the other TCR loci, perhaps the irradiation effect does not persist long enough. Many rounds of cell division are initiated at the DN–DP transition, and the irradiation-induced change in DNA repair potential may not be maintained through many cell cycles. This is consistent with the observation that induced radioresistance persists in fibroblasts for only three cell cycles (29). A second, nonexclusive possibility is based on the observation that successful rearrangements at the other TCR loci, but not TCR-α, can undergo substantial amplification during the proliferation that accompanies the DN–DP transition. Thus, rescue of all loci may actually be rather inefficient; yet proliferation of cells that contain rare successful rearrangements amplifies the rescued rearrangements at the other loci but not at TCR-α. This hypothesis is supported by two observations: (a) >90% of rescued TCR-β rearrangements are in frame (5) and (b) rare TCR-α rearrangements have been detected in irradiated SCID thymocytes RNA by reverse transcriptase–PCR (5), and we have repeatedly detected very low levels of TCR-α rearrangements in irradiated SCID thymocytes (8). These could result from irradiation-mediated rescue of TCR-α recombination without the benefit of selective amplification provided by the proliferation that accompanies the DN–DP transition.

A second question raised by our results is the failure of rescue to occur in the B cell lineage, an issue that is especially perplexing if irradiation is indeed inducing DNA repair activities in a lymphoid progenitor cell capable of giving rise to either B or T lymphocytes. We have considered several possible explanations. First, the SCID or RAG/NK hosts may lack appropriate niches in the bone marrow stroma, so that precursor cells cannot proceed with their differentiation program. Another possibility is that the cells in the B cell lineage may fail to proliferate sufficiently after irradiation to allow detection of rescued rearrangements. Studies in RAG-/- mice, which are incapable of V(D)J rearrangement, have shown that irradiation provides a signal for proliferation and differentiation that appears to be thymocyte specific (22). This signal may be required for expansion of precursor lymphocytes containing rescued rearrangements. Further experiments will be required to answer these interesting questions.
References

1. Shortman, K., and L. Wu. 1996. Early T lymphocyte progenitors. Annu. Rev. Immunol. 14:29–47.

2. Godfrey, D.I., J. Kennedy, T. Suda, and A. Zlotnik. 1993. A developmental pathway involving four phenotypically and functionally distinct subsets of CD3-CD4-CD8- triple-negative adult mouse thymocytes defined by CD44 and CD25 expression. J. Immunol. 150:4244–4252.

3. Wu, L., R. Scollay, M. Antica, G.R. Johnson, R. Scollay, and K. Shortman. 1994. Rescue of T cell-specific V(D)J recombination in SCID mice by DNA damaging agents. Science. 266:450–455.

4. Bogue, M., C. Zhu, E. Aguilar-Cordova, L.A. Donehower, and D.B. Roth. 1996. p53 is required for both radiation-induced differentiation and rescue of V(D)J rearrangement in scid mouse thymocytes. Genes Dev. 10:553–565.

5. Zhou, C., M.A. Bogue, and D.B. Roth. 1996. Thymocyte differentiation in gamma-irradiated severe-combined immunodeficiency mice: characterization of intermediates and products of V(D)J recombination at the T cell receptor (α locus. Eur. J. Immunol. 26:2859–2865.

6. Livak, F., S.C. Welsh, C.J. Guidos, I.N. Crispe, I.N. Crispe, J.S. Danska, and D.G. Schatz. 1996. Transient restoration of gene rearrangement at multiple T cell receptor loci in gamma-irradiated scid mice. J. Exp. Med. 184:419–428.

7. Kadish, J.L., and R.S. Basch. 1975. Thymic regeneration after lethal irradiation: evidence for an intra-thymic radioresistant T cell precursor. J. Immunol. 114:452–458.

8. Tomooka, S., G. Matsuzaki, K. Kishihara, K. Tanaka, Y. Yoshikai, K. Taniguchi, K. Himeono, and K. Nomo. 1987. Sequential appearance of thymocyte subpopulations and T cell antigen receptor gene messages in the mouse thymus after sublethal irradiation. J. Immunol. 139:3986–3990.

9. Wite, L., Z. Fuks, A. Hamovitz-Friedman, I. Vinogradsky, D.S. Goodman, and A. Eldor. 1989. Effects of irradiation on the release of growth factors from cultured bovine, porcine, and human endothelial cells. Cancer Res. 49:5066–5072.

10. Weiselbaum, R.R., D.E. Hallahan, V. Sukhatme, A. Dritschild, M.L. Sherman, and D.W. Kufe. 1991. Biological consequences of gene regulation after ionizing radiation exposure. J. Natl. Cancer Inst. 83:480–484.

11. Neta, R., and J.J. Opperhain. 1991. Radiation protection with cytokines—learning from nature to cope with radiation damage. Cancer Res. 51:391–396.

12. Shore, E.W., S.O. Sharrow, I. Uppenkamp, and A. Singer. 1990. T cell receptor-negative thymocytes from SCID mice can be induced to enter the CD4/CD8 differentiation pathway. Eur. J. Immunol. 20:69–77.

13. Lynch, F., and E.M. Shevach. 1993. γδ T cells promote CD4 and CD8 expression by SCID thymocytes. Int. Immunol. 5:991–995.

14. Goldscheider, I., K.L. Komschlies, and D.L. Greiner. 1986. Studies of thymocyteopoiesis in rats and mice. I. Kinetics of appearance of thymocytes using a direct intrathymic adoptive transfer assay for thymocyte precursors. J. Exp. Med. 163:1–17.

15. Garman, R.D., P.J. Doherty, and D.H. Rauler. 1986. Diversity, rearrangement, and expression of mouse T cell gamma genes. Cell. 45:733–742.

16. Hsu, J.S., J. Anderson, M.C. Louie, S.A. Godambe, M.R. Pozzi, M.A. Behlke, K. Huppi, and D.Y. Loh. 1987. Tandem linkage and unusual RNA splicing of the T-cell receptor β-chain variable-region genes. Proc. Natl. Acad. Sci. USA. 84:1992–1996.

17. Maligne, M., K. Minard, S. Molsness, M. Kronenberg, J. Goverman, T. Humplki, M.B. Prystowsky, Y. Yoshikai, F. Fitch, T.W. Mak, et al. 1984. Mouse T cell antigen receptor: structure and organization of constant and joining gene segments encoding the β polypeptide. Cell. 7:1101–1110.

18. Aguilar, L.f., and J.W. Belmont. 1991. Vγ3 T cell receptor rearrangement and expression in the adult thymus. J. Immunol. 146:1348–1352.

19. Guidos, C.J., C.J. Williams, G.E. Wu, C.J. Paige, and J.S. Danska. 1995. Development of CD4+CD8- thymocytes in Rag-deficient mice through a T cell receptor β-chain-independent pathway. J. Exp. Med. 181:1187–1195.

20. Antica, M., L. Wu, K. Shortman, and R. Scollay. 1994. Thymic stem cells in mouse bone marrow. Blood. 84:111–117.

21. Kondo, M., I.L. Weissman, and K. Akashi. 1997. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell. 91:661–672.

22. Wu, L., M. Antica, G.R. Johnson, R. Scollay, and K. Shortman. 1991. Developmental potential of the earliest precursor cells from the adult mouse thymus. J. Exp. Med. 174:1617–1627.

23. Soloff, T.-G., L. Lybarger, D. Dempsey, and R. Chervenak. 1996. Pre-thymic transcription of TCR genes by adult murine bone marrow cells. Mol. Immunol. 33:957–964.

24. Scollay, R.S., T.-G. Wang, L. Lybarger, D. Dempsey, and R. Chervenak. 1995. Transcription of the TCR-β locus initiates in adult murine bone marrow. J. Immunol. 154:3888–3901.

25. Arpes, B., P. Lambin, K.A. Skov, and M.C. Joiner. 1997. Low dose hyper-radiosensitivity and increased radioresistance in mammalian cells. Int. J. Radiat. Biol. 71:721–735.

26. Shadle, J.D., V. Afzal, and S. Wolff. 1987. Characterization of the adaptive response to ionizing radiation induced by low doses of x-rays to human lymphocytes. Radiat. Res. 111:511–517.

27. Morgan, W.F., J.P. Day, M.I. Kaplan, E.M. McGhee, and C.L. Limoli. 1996. Genomic instability induced by ionizing radiation. Radiat. Res. 146:247–258.

28. Mador, M., R.J. Antonioni, and J.L. Reed. 1993. Delayed heritable damage and epigenetics in radiation-induced neoplastic transformation of human hybrid cells. Radiat. Res. 134:209–216.