Induction of Apoptosis and Expression of Apoptosis-related Gene Products in Response to Radiation in Murine Tumors

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Purpose : To analyze the involvement of apoptosis regulatory genes p53, $p21^{waf1/cip1}$, bax and bcl-2 in induction of apoptosis by radiation in murine tumors.

Materials and methods : The radiation-sensitive ovarian carcinoma OCa-I, and the radiation-resistant hepatocarcinoma HCa-I were used. Tumors, 8 mm in diameter, were irradiated with 25 Gy and at various times after irradiation, ranging from 1 to 48 h, were analyzed histologically for apoptosis and by western blot for alterations in the expression of these genes. The p53 status of the tumors were determined by the polymerase chain reaction-single strand conformation polymorphism assay.

Results: Both tumors were positive for wild-type p53. Radiation induced apoptosis in OCa-I but not in HCa-I. Apoptosis developed rapidly, peaked at 2 h after irradiation and returned to almost the background level at 48 h. In OCa-I radiation upregulated the expression of p53, $p21^{waf1/cip1}$, and the bcl-2/bax ratio was decreased. In HCa-I radiation increased the expression of both p53 and $p21^{waf1/cip1}$, although the increase of the latter was small. The bcl-2/bax ratio was greatly increased. In general the observed changes occurred within a few hours after irradiation, and either preceded or coincided with development of apoptosis.

Conclusions : The development of apoptosis required upregulation of both p53 and $p21^{waf1/cip1}$ as well as a decrease in bcl-2/bax ratio. In contrast, an increase in bcl-2/bax ratio prevented apoptosis in the presence of upregulated p53 and $p21^{waf1/cip1}$. These findings indentified the involvement of multiple oncogenes in apoptosis regulation in vivo and demonstrate the complexity that may be associated with the use of a single oncogene assessment for predicting the outcome of cancer therapy with cytotoxic agents.

Key Words : Apoptosis, Murine tumors, Radiation, p53, $p21^{waf1/cip1}$, Bax and bcl-2

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INTRODUCTION

Apoptosis is, in addition to reproductive cell death, a major mode of cell killing by ionizing radiation both in normal tissues and tumors[1-3]. It develops rapidly, within hours, after radiation, and is dose dependent[4, 5]. Radiation-induced apoptosis varies among different tumors but it positively correlates with the antitumor efficacy of radiation[6], which makes apoptosis a potential predictor of tumor treatment outcome after radiotherapy[7, 8]. Also, the regulation of apoptosis induction may be used for the improvement of radiotherapy through either increasing apoptotic response of tumors or through inhibiting apoptotic response of normal tissues.

Apoptosis, both as a physiological mechanism of cell deletion or induced by cytotoxic agents, is regulated by a number of genes such as p53, $p21^{waf1/cip1}$, bax and bcl-2[9-12]. Tumor suppressor gene p53 maintains normal cell growth by controlling the transition of cells through the G1/S phase checkpoint of the cell cycle[12], and is an important determinant in apoptosis induction by radiation and a number of other cytotoxic agents[12-14]. Cells expressing wild-type p53 protein were found to be sensitive to apoptosis induction, whereas cells lacking p53 or containing mutant forms of this gene were resistant[12, 14]. After genetoxic insult, p53 can activate effector genes such as $p21^{waf1/cip1}$, a potent inhibitor of cyclin dependent kinases[15, 16]. This gene is primarily responsible for G1 arrest, but is also involved in inducing apoptosis[16]. In contrast to these two genes, bcl-2 inhibits apoptosis[9] and through this process is involved in promotion of oncogenesis[17, 18], and in rendering tumor cells resistant to cytotoxic agents[19, 20]. It has been postulated that bcl-2 inhibits apoptosis by forming heterodimers with the apoptosis promoting gene bax, and that the ratio of bax and bcl-2 proteins determine relative susceptibility of cells to apoptosis[11, 21-22]. Most information on the involvement of these oncogenes in apopotis induction comes from in vitro studies using well-defined cell lines and highly controlled micorenvironmental conditions[14]. The involvement of individual genes varied among cell lines used and apoptotic response was in addition frequently dependent on the presence of growth factors or cytokines[14, 23-24].

Studies on the involvement of apoptosis-regulating genes in the in vivo tumor response to cytotoxic agents are still limited. Most information is related to the dependency of cancer treatment outcome on tumor p53 status[14]. Although many reports showed that p53 mutation is associated with poorer treatment outcome, the results in general are still equivocal and may reflect wide heterogeneity in the p53 response depending on tumor type, anticancer agent used and involvement of other oncogenes[14]. That p53 has a beneficial role in tumor therapy was recently demonstrated by transferring wild-type p53 to cells of tumor xenografts[25] or to tumors in humans[26]. The transfected cells underwent apoptosis resulting in tumor regression.

In a study of the effects of radiation on induction of apoptosis in murine tumors we observed that one third of irradiated tumors (5/15) responded by significant apoptosis which correlated with the efficacy of radiation to delay tumor growth and cure tumors[6]. One sensitive tumor, mammary carcinoma MCa-4, and one resistant tumor, squamous cell carcinoma SCC-VII, were analyzed for p53 and $p21^{waf1/cip1}$ expression after irradiation[27]. Both tumors contained w-t p53, and both exhibited p53 protein upregulation after radiation. Since radiation upregulated $p21^{waf1/cip1}$ only in MCa-4 tumor, we concluded that upregulation of both p53 and $p21^{waf1/cip1}$ was necessary for induction of apoptosis by radiation. In the present study we extended this investigation to two additional murine tumors, an apoptosis sensitive ovarian carcinoma and an apoptosis resistant hepatocarcinoma, to determine the influence of p53, $p21^{waf1/cip1}$, bax and bcl-2 oncogenes on radiation-induced apoptosis.

MATERIAL AND METHODS

1. Mice and tumors

Male C3Hf/Kam mice, bred and maintained in our specific-pathogen-free mouse colony, were 4 months old at the beginning of the experiments and housed 5 in each cage. The tumors used were an ovarian adenocarcinoma, designated OCa-I, and a hepatocarcinoma, designated HCa-I, syngeneic to and nonimmunogenic in this strain of mice. OCa-I was used in its 7th and HCa-I in its 5th isotransplant generation. The former tumor is sensitive and the latter resistant to ionizing radiation as assessed by tumor growth delay[4] and tumor radiocurability[28]. Solitary tumors were produced in the muscles of the right thigh by the inoculation of 5×10^5 cells. Tumor cell suspensions were prepared by mechanical disruption and enzymetic digestion of non-necrotic tumor tissue[29].

2. Tumor irradiation

Tumors were locally irradiated when they grew to 8 mm in mean diameter. During irradiation the unanesthetized mice were immobilized in a jig and tumors centered in a 3 cm diameter circular field. A single dose of 25Gy γ-radiation was delivered, using a dual source ^{137}Cs unit, at a dose rate of 6.5 Gy/min. The effect of radiation on tumor growth was determined by measuring three orthogonal tumor diameters with Vernier calipers at 2 day intervals until tumors grew to at least 12 mm in diameter.

3. Analysis of apoptosis

Mice were killed by cervical dislocation at different times after irradiation, ranging from 1 to 48 h, and the tumors were immediately excised and placed in neutral buffered formalin. The tissues were embedded in paraffin blocks and 4 μm sections were cut from these and stained with hematoxylin and eosin (H&E). The apoptotic cells were scored in coded slides at 400×magnification. The morphological features used to identify apoptosis in murine tumors have been previously described and illustrated[5, 30]. Five fields of nonnecrotic areas were selected randomly across each tumor section, and in each field apoptotic bodies were expressed as a percentage based on the scoring of 1500 nuclei (2000 nuclei for untreated controls) at each time interval after treatment.

4. Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP)

PCR-SSCP analysis was performed to screen for the presence of p53 mutation in tumors. PCR of p53 gene exons 5 through 8 were performed on fresh-frozen untreated tumors and wild-type p53 (control). The primers used were as follows: Exon 5, 5'TCTCTTCCAGTACTCTCCTC3', 5'GAGG GCTTACCATCACCATC3': Exon 6, 5'TTGCTCTAA GGCCTGGCTCC3', 5'AATTACAGACCTCGGGTGG-C3'; Exon 7, 5'TCTTCCCCAGGCCCGGCTCTCG 3', 5'GCTTTCCTACCTGGA-GTCTT3'; Exon 8, 5'T CCCGGATAGTGGGAACCTT3', 5'GCCTGCGTA-CC TCTCTTTGC3'; PCR was carried out in a DNA Thermal Cycler (Perkin-Elmer Cetus) for 30 cycles, each consisting of a denaturing phase of 1 min at 94℃ for 10 min to denature DNA and place on ice to prevent renaturation. Finally, a 6 μl sample was loaded onto an MDE gel (FMC Bio. Pro., ME), electrophoresed at 30 W for 4-5 h in 8 ℃ before drying and exposed to X-ray film at -70 ℃ overnight.

5. Western blot analysis

Tumor tissues were collected from tumor-bearing mice at different times ranging from 1 to 48 h after radiation. Approximately 1 mm^3 of tissues were diced into very small pieces using a clean razor blade. Tissues were washed 3 times in ice-phosphate buffered saline (PBS, pH 7.4) and lysed on ice for 1 h in 0.5% NP40 prepared in Tris buffered saline (TBS) that contained 1 μl/ml of dithiothereitol (DTT, Sigma), 10 μl/ml of PMSF (Sigma), 20 mM Tris-Hydrochloride (pH 7.6), 150 mM sodium chloride and 5mM EDTA. The samples were centrifuged at 4℃ for 20 min, and supernatants were transferred into new tubes. The

samples were then frozen and stored at -7℃. Samples were thawed, diluted in 2×SDS-polyacrylamide gels. The protein bands were then transferred electrophoretically to nitrocellulose membranes (Biorad). After transfer was completed, the gels were stained with Comassie blue to verify equal sample loading. Blots were blocked by incubation with PBS containing 0.1% tween-20 (PBST) and 5% nonfat milk for 2 h at room temperature. The blots were then incubated for 2 h at room temperature in each primary antibody including p53 (Ab 7, Oncogene Science, Cambridge MA), $p21^{waf1/cip1}$ (Ab 5, Oncogene Science), bcl-2 (Ab 7, Oncogene Science), and bax (p-19, Santa Cruz Biotenchnology Inc., Santa Cruz, CA). After washing in PBS containing 0.1% Tween-20, and the blots were subsequently incubated for 1 h at room temperature in either an anti-mouse or anti-rabbit anti-IgG antibody conjugates (Amersham International, Little Chalfont, UK), which in the presence of horseradish peroxidase released chemiluminescence that was detected on X-ray film. Quantitative analysis of detected proteins was carried out using DU-70 Spectrophotometer (Beckman Instrument Ind., Fullerton, CA).

RESULT

1. Apoptosis induction

Fig. 1 shows apoptosis induction in OCa-I and HCa-I tumors as a function of time, ranging from 1 to 48 h, after 25 Gy tumor irradiation. Untreated OCa-I tumors contained 1.8±0.6% and HCa-I tumor 0.2±0.0% apoptotic cells. Radiation rapidly induced apoptosis in OCa-I which peaked already at 2 h after the treatment at which time 16.1± 0.6% cells were apoptotic. After this the percentage of apoptotic cells declined, remained elevated 1 day later and approached the background level 2 days after irradiation. In contrast to its effect on OCa-I, radiation was totally ineffective in inducing apoptosis in HCa-I, findings similar to those of our earlier studies[4, 5]. The insert in Fig. 1 shows that after 25 Gy the apoptosis sensitive OCa-I exhibited rapid partial regression and long

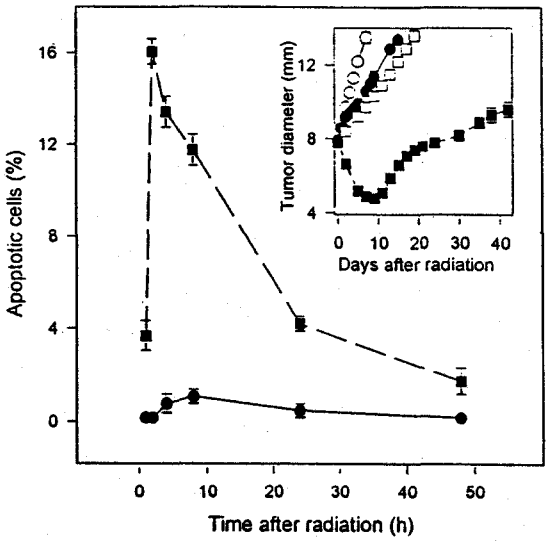

Fig. 1. Induction of apoptosis in OCa-I (squares) and HCa-I (circles) murine tumors as a function of time following 25 Gy local tumor irradiation. Tumors were 8 mm at the time of irradiation. Vertical bars, SE of the mean. In the insert are growth curves of OCa-I (solid symbols) and HCa-I (open symbols), untreated (circles) or treated with 25 Gy (squares).

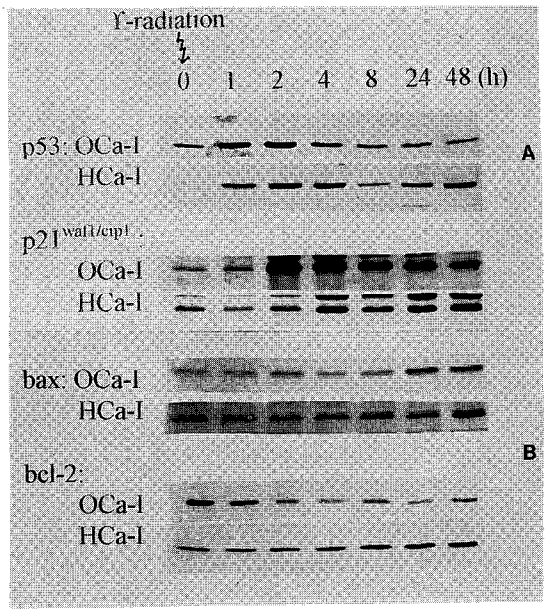

Fig. 2. Western blots of p53 and $p21^{waf1/cip1}$ proteins (A) and bcl-2 and bax proteins (B) in OCa-I and HCa-I murine tumors as a function of time following 25 Gy local tumor irradiation

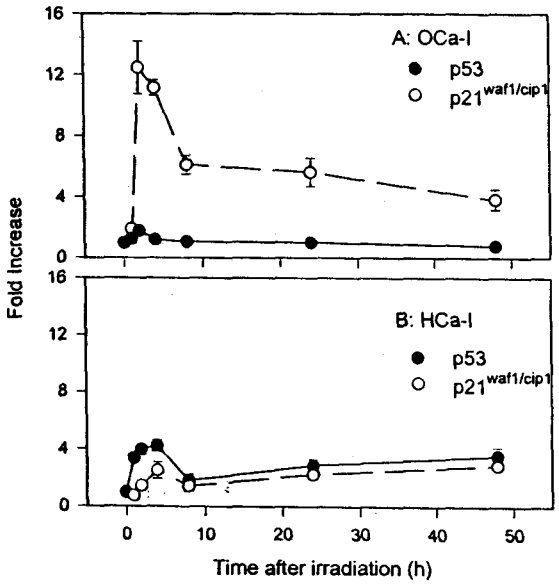

Fig. 3. Expression in p53 (solid) and $p21^{waf1/cip1}$ (open) proteins in OCa-I (A) and HCa-I (B) murine tumors as a function of time following 25 Gy local tumor irradiation. Tumors were 8 mm at the time of irradiation. Vertical bars, SE of the mean.

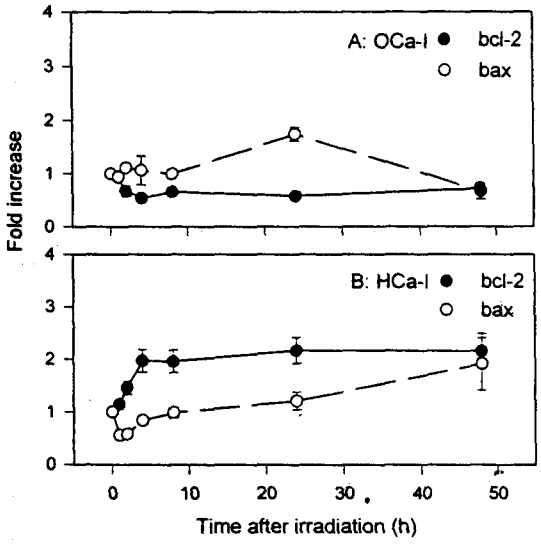

Fig. 4. Expression in bax (open) and bcl-2 (solid) proteins in OCa-I (A) and HCa-I (B) murine tumors as a function of time following 25Gy local tumor irradiation. Tumors were 8mm at the time of irradiation. Vertical bars, SE of the mean.

growth delay, whereas the apoptosis resistant HCa-I showed only a small tumor growth delay that began several days after irradiation.

2. Alteration in oncogene expression

OCa-I and HCa-I tumors were analyzed for p53, $p21^{waf1/cip1}$, bax, bcl-2 expression at the same time points after 25 Gy at which apoptosis induction was determined (Fig. 2-4). Radiation induced a rapid increase in p53 protein level in OCa-I which peaked 1 h after irradiation when the amount of protein was 1.9 times higher than the control value (Fig. 3A). The p53 returned to the baseline level several hours later. The amount of $p21^{waf1/cip1}$ protein also increased rapidly after radiation reaching its peak 2 h after radiation delivery at which time the increase was 12.5-fold the control value (Fig. 3A). It then decreased, first rapidly by 8 h after irradiation and then slowly until 48 h after the treatment when it was still 3.8 times above the background level. The expression of bax protein underwent no significant change at any of the time points after irradiation with the exception of the 24 h point when the expression was 1.7 times higher than the control value (Fig. 3A). In contrast to its effect on the above oncogenes, radiation caused a decrease in bcl-2 protein expression (Fig. 4A). The nadir of 0.5 the control value was reached 4 h after irradiation at which level bcl-2 remained until the end of the observation period of 48 h. The ratio of bcl-2 over bax was calculated and plotted in Fig. 5; it was significantly decreased between 2 h and 48 h after irradiation.

In HCa-I tumor, p53 protein level was also increased after irradiation (Fig. 3B). The increase was greater than that in OCa-I but it reached its peak later, at 4 h after irradiation, when the level was 4.2 times higher than the control value. After than p53 protein level decreased but still remained elevated throughout the remaining observation period. Radiation also increased $p21^{waf1/cip1}$ protein levels the kinetics of which were almost identical to that of p53 with a difference that the increase began after than of p53 (Fig. 3B). However, the

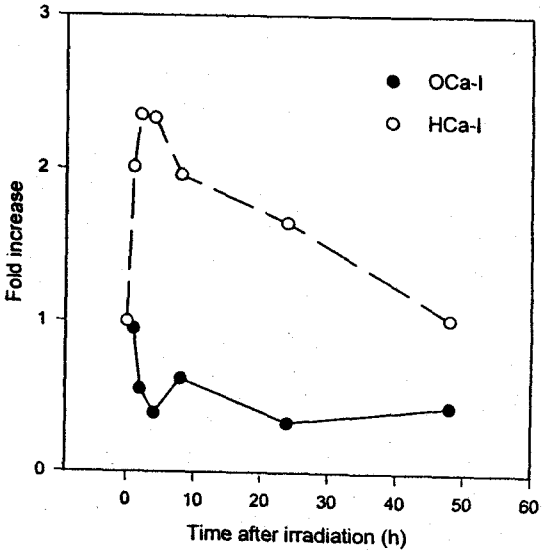

Fig. 5. Ratio of bcl-2 over bax proteins in OCa-I (solid) and HCa-I (open) murine tumors as a function of time following 25 Gy local tumor irradiation. Tumors were 8 mm at the time of irradiation.

increase in $p21^{waf1/cip1}$ was smaller than that of p53; it was higher than the control value by a factor of 2.5 at its peak 4 h after irradiation. Radiation caused rapid but transient decrease in bax protein, the nadir of which was at 1 h after irradiation when the bax level was reduced by a factor of 0.6 (Fig. 4B). The expression of bax returned to normal at 4 h after irradiation when it started to increase slowly and at 48 h after irradiation was 1.9 times higher than the control value. Radiation increased bcl-2 protein expression that began 1 h after the treatment and reached its plateau of 2.2-fold the control value that lasted throughout the observation period (Fig. 4B). Fig. 5 plots the ratio of bcl-2 over bax; the ratio was increased significantly at all time points, being the highest 2 h after irradiation when it was 2.5. OCa-I and HCa-I tumors were analyzed for mutations of p53 in exons 5 through 8 using PCR-SSCP method. No evidence of a p53 mutation was identified.

DISCUSSION

The results obtained in this study showed that cellular and molecular events after irradiation greatly differed between radiosensitive OCa-I and radioresistant HCa-I tumors. The cellular events assayed consisted of apoptosis induction, whereas the molecular parameters were p53, $p21^{waf1/cip1}$, bax and bcl-2 expression. A 25 Gy single dose irradiation caused rapid but transient regression of OCa-I resulting in a long delay in tumor regrowth but only a slight growth delay of HCa-I tumor beginning to be noticeable several days after irradiation. At the cellular level irradiated OCa-I exhibited significant apoptosis, while HCa-I displayed complete absence of apoptosis. Radiation-induced apoptosis developed rapidly, being significantly elevated already 1 h after irradiation, peaked at 4 h after irradiation and was still above the background level two days later (Fig. 1). Because apoptotic cells disappear within 3 h of their induction[31] the total tumor cells loss by apoptosis in OCa-I after irradiation must have been high to account for the observed rapid tumor regression and long delay in tumor regrowth (Fig. 1). On the other hand, the radioresistance of HCa-I tumor could be attributed to the lack of apoptosis induction.

There is ample evidence that apoptosis induced by DNA-damaging agents, including radiation, is regulated by oncogenes, primarily by p53. In general, it is thought that w-t p53 is needed for the induction of apoptosis whereas the loss or mutation of this gene would results in resistance to apoptosis induction[12-14]. Both tumors used in the present study, as well as the apoptosis sensitive MCa-4 tumor and the apoptosis resistant SCC-VII tumor used in our earlier study[27], were w-t p53 tumors. Based on the results obtained with the 4 tumors, it is clear that murine solid tumors possessing w-t p53 can be both sensitive and resistant to radiation, and that their sensitivity is likely to depend not only on the activation of p53 in tumor cells but also on some downstream

events regulating apoptosis, such as the involvement of $p21^{waf1/cip1}$, bax or bcl-2.

In a previous study[27] we demonstrated that radiation-induced apoptosis required activation of both p53 and $p21^{waf1/cip1}$. The resistant SCC-VII tumor responded to radiation by a strong p53 expression but with no change in the expression of $p21^{waf1/cip1}$. The results in Fig. 3 show that radiation increased the expression of both p53 and $p21^{waf1/cip1}$ in both apoptosis-sensitive OCa-I and apoptosis-resistant HCa-I tumors although the magnitude of the increase was different between the two tumors. The amount of p53 protein increased more in HCa-I (4.2-fold) than in OCa-I (1.9-fold) while the amount of $p21^{waf1/cip1}$ protein increased more in OCa-I (12.5-fold) than in HCa-I (2.5-fold). It is possible that these quantitative differences in the magnitude of oncogene expression influenced apoptotic process. Zhang et al[32] recently reported that the inhibition of cyclin/cdk activation by $p21^{waf1/cip1}$ depended on the absolute level of $p21^{waf1/cip1}$. When the level of $p21^{waf1/cip1}$ in irradiated cells was low the cells failed to adequately arrest in G1 and to undergo apoptosis. Here, high level of induced $p21^{waf1/cip1}$ protein in HCa-I tumor was associated with the lack of apoptosis induction. Thus, either the lack[27] or a low level of $p21^{waf1/cip1}$ activation (Fig. 3) in the presence of activated p53 were associated with the inability of radiation to induce apoptosis.

The present study also assessed the effects of radiation on the expression of bax and bcl-2 oncogenes whose actions may depend on p53. P53 was reported to induced rapid elevation in the expression of bax[33]. The relevance of bax to radiation-induced apoptosis was recently studied using several tumor cell culture lines[34]. The cell lines that possessed w-t p53 exhibited upregulation of p53 and cell cycle arrest after irradiation; however, radiation induced apoptosis primarily in cell lines in which the upregulation of bax also occurred. We observed here only small changes in the expression of bax after irradiation: A slight increase in OCa-I and a very rapid but transient decrease in HCa-I. In comparison, the changes in bcl-2 expression after irradiation were more profound. Bcl-2 decreased in OCa-I and increased in HCa-I. However, the blc-2/bax ratio was much different between the two tumors after irradiation. The ratio decreased in the radiosensitive OCa-I and increased in the radioresistant HCa-I. Our observation is in accord with earlier findings, obtained with in vitro cell lines, that change in ratio between these two oncogenes rather than the absolute change of each individually determines whether apoptosis will occur[11, 21-22]. The increased bcl-2/bax ratio is associated with induction whereas decreased ratio is associated with the lack of apoptosis[11, 21-22]. It has been postulated that bcl-2 inhibits apoptosis by forming dimers with bax and by blocking the apoptotic action of bax homodimers[22, 35].

The results of our previous[27] and the present study showed that murine tumors possessing w-t p53 can be sensitive or resistant to apoptosis induction by radiation, which in turn manifested in greater or poorer tumor growth delay (Fig. 1) or tumor radiocurability[28], in spite of p53 being upregulated by radiation. For apoptosis to occur $p21^{waf1/cip1}$ was required to be upregulated as well and the bcl-2/bax ratio had to decrease. In the resistant tumor, the upregulation of $p21^{waf1/cip1}$ was either small or lacking and bcl-2/bax ratio increased to favor apoptosis-inhibitory actions of bcl-2. An important aspect of our study is that the information was obtained using in vivo tumors, where the efficacy of cytotoxic treatments, including radiation, is determined by multiple genetic and environmental factors. These findings along with those reported earlier for paclitaxel[27] show that it is possible to quantify the expression of different oncogenes in tumors after cytotoxic insult and relate the induced changes to the efficacy of tumor therapy. However, they demonstrate the complexity associated with using a single oncogene and a single time point assessment in order to predict treatment outcome since the effect of cytotoxic agents may depend on activation of more than one oncogene which may be activated at different times after the treatment.

REFERENCES

1. **Milas L, Stephens LC, Meyn RE.** Relation of apoptosis to cancer therapy. In vivo 1994; 8: 665-674
2. **Dewey WC, Ling CC, Meyn RE.** Radiation-induced apoptosis: Relevance to radiotherapy. Int J Rad Oncol Biol Phys 1995; 33:781-796
3. **Meyn RE, Stephens LC, Milas L.** Programmed cell death and radioresistance. Cancer and Metastasis Rev 1996; 15:119-131
4. **Stephens LC, Ang KK, Schultheiss TE, et al.** Apoptosis in irradiated tumors. Rad Res 1991;127: 308-317
5. **Stephens LC, Hunter NR, Ang KK, et al.** Development of apoptosis in irradiated murine tumors as a funciton of time and dose. Rad Res 1993; 135:75-80
6. **Meyn RE, Stephens LC, Ang KK, et al.** Heterogeneity in the development of apoptosis in irradiated murine tumors of different histologies. Int J Radiat Biol 1993; 64:583-591
7. **Wheeler JA, Stephens LC, Tornos C, et al.** ASTRO research fellowship: Apoptosis as a predictor of tumor response to radiation in state IB cervical carcinoma. Int J Rad Oncol Biol Phys 1995; 32:1487-1493
8. **Chyle V, Pollack A, Czerniak BA, et al.** Apoptosis and downstaging after preoperative radiotherapy for muscle-invasive bladder cancer. Int J Rad Oncol Biol Phys 1996; 35:281-287
9. **Korsmeyer SJ.** Bcl-2: a repressor of lymphocyte death. Immunol Today 1992; 13:285-288
10. **Wyllie AH.** Apoptosis and the regulation of cell numbers in normal and neoplastic tissues: an overviews. Cancer and Metastasis Rev 1992; 11:95-103
11. **Oltavi ZN, Milliman CL, Korsmeyer SJ.** Bcl-2 heterodimerizes in vivo with a conserved homolog, bax, that accelerates programmed cell death. Cell 1993; 74:609-6190
12. **Kastan MB, Canman CE, Leonard CJ.** p53, cell cycle control and apoptosis: Implications for cancer. Cancer and Metastasis Rev 1995; 14:3-15
13. **Lowe S, Bodis S, McClatchey A, et al.** p53 status and the efficacy of cancer therapy in vivo. Science 1994; 266:807-810
14. **Harris CC.** Structure and function of p53 tumor suppressor gene: Clues for rational cancer therapeutic strategies. J Natl Cancer Inst 1996; 88: 1442-1455
15. **Harper JW, Adami GR, Wei N, et al.** The p21 cdk interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 1993; 75:805-816
16. **El-Deiry WS, Harper JW, O'Conner PM, et al.** WAF1/CIP1 Is induced in p53-mediated G1 arrest and apoptosis. Cancer Res 1994; 54:1169-1174
17. **Reed J, Haldar S, Cuddy M, et al.** Bcl-2 mediated tumorigenecity in a T-lymphoid cell line: synergy with c-myc and inhibition by bcl-2 antisense. Proc Natl Acad Sci USA 1990; 87:3660-3664
18. **Marin MC, Hsu B, Meyn RE, et al.** Evidence that p53 and bcl-2 are regulators of a common cell death pathway important for in vivo lymphomagenesis. Oncogene 1994; 9:3107-3112
19. **Carson DA and Ribeiro JM.** Apoptosis and disease. Lancet 1993; 341:1251-1254
20. **Kitada S, Takayama S, Dariel K, et al.** Reversal of chemoresistance of lymphoma cells by antisense-mediated reduction of bcl-2 gene expression. Antisense Res Dev 1994; 4:71-79
21. **Korsmeyer SJ, Shutter JR, Veis DJ, et al.** Bcl-2/Bax: a rheostat that regulates an antioxidant pathway and cell death. Cancer Biol 1993; 4:327-332
22. **Yang E, Zha J, Jockel J, et al.** Bad: a heterodimertic partner for bcl-X_L and bcl-2, displaces bax and promotes cell death. Cell 1995; 80:285-291
23. **Willye AH, Rose KA, Morris RG, et al.** Rodent fibroblast tumors expressing myc and ras genes: growth, metastasis and endogenous oncogene expression. Brit J Cancer 1987; 56:251-259
24. **Canman CE, Gilmer TM, Coutts SB, Kastan MB.** Growth factor modulation of p53-mediated growth arrest versus apoptosis. Genes & Dev 1995; 9:600-611
25. **Liu TJ, Zhang WW, Taylor DL, et al.** Growth suppression of human head and neck cancer cells by the introduction of a wild-type p53 gene via a recombinant retrovirus. Cancer Res 1994; 54:3662-3667
26. **Roth JA and Cristiano RJ.** Gene therapy for cancer: what have we done and where are we going? J Natl Cancer Inst 1996; 88:21-39
27. **Saito Y, Milross CG, Hittelman WN, et al.** Effect of radiation and paclitaxel on p53 expression in murine tumors sensitive or resistant to apoptosis induction. Int J Radiat Oncol Biol Phys in press
28. **Milas L, Wike J, Hunter N, et al.** Macrophage content of murine sarcomas and carcinomas: associations with tumor growth parameters and

tumor radiocurability. Cancer Res 1987; 47:1069-1075

29. **Milas L, Hunter N, Mason K, et al.** Immunological resistance to pulmonary metastases in C3Hf/Bu mice bearing syngeneic fibrosarcoma of different sizes. Cancer Res 1974; 34:61-71
30. **Milas LM, Hunter NR, Kurdoglu B, et al.** Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with Taxol (paclitaxel). Cancer Chemother Pharmacol 1995; 325:297-303
31. **Busch W, Paffe S, Putx B, et al.** Determination of the length of the histological stages of apoptosis in normal liver and in altered hepatic foci of rats. Carcinogenesis 1990; 11:847-853
32. **Zhang H, Hannon GJ, Beach D.** p21-containing cyclin kinases exist in both active and inactive states. Genes and Dev 1994; 8:1750-1758
33. **Miyashita T, Krajewski S, Krajewska M, et al.** Tumor supressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. Oncogene 1994; 9:1799-1805
34. **Zhan Q, Fan S, Bae I, et al.** Induction of bax by genotoxic stress in human cells correlates with normal p53 status and apoptosis. Oncogene 1994; 9:3743-3751
35. **Sato T, Hanada M, Bodrag S, et al.** Interaction among members of the bcl-2 protein family analyzed with a yeast two-hybrid system. Proc Natl Aca Sci USA 1994; 91:9238-9242

= 국문 초록 =

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목 적 : 세포 독성 인자가 유도하는 아포프토시스에 관한 연구가 대부분 in vitro 연구에 국한되어온 바, in vivo에서 방사선에 의한 아포프토시스의 유도와 이에 관여하는 유전자들의 발현 양상을 분석하기 위하여 본 연구를 수행하였다.

대상 및 방법 : 마우스 동종암으로서 방사선 민감 종양인 난소암 (OCa-I)과 내성 종양인 간암 (HCa-I)을 모델로 하여 이들 종양이 평균 직경 8 mm로 자랐을 때 25 Gy의 방사선을 조사하였다. 조사 후 다양한 시간 간격으로 조직을 채취하여 아포프토시스의 유도 수준을 분석하며 동시에 이에 관련된 유전자 산물인 p53, $p21^{waf1/cip1}$, bax, bcl-2 등의 발현을 western blotting 을 이용하여 분석하였다. 종양의 p53 상태는 polymerase chain reaction-single strand conformation polymorphism assay로 분석하였다.

결 과 : 모델 종양들의 p53 상태는 둘 다 자연형으로 나타났다. 방사선 조사로 OCa-I에서는 아포프토시스가 유도되었으나 HCa-I에서는 아포프토시스가 관찰되지 않았다. OCa-I에서 방사선 조사로 p53, $p21^{waf1/cip1}$의 발현이 증가되었으며 bcl-2/ bax 비율은 감소하였다. HCa-I에서는 p53, $p21^{waf1/cip1}$의 발현이 증가되었으나 $p21^{waf1/cip1}$은 OCa-I과 비교하여 증가 수준이 미약하였다. bcl-2/bax 비율은 현저히 증가하였다. 이와 같은 변화들은 방사선 조사에 선행되거나 조사 후 수 시간 내에 일어났으며 아포프토시스의 유도에 선행하거나 일치하였다.

결 론 : 아포프토시스의 진행에는 p53, $p21^{waf1/cip1}$의 증가 뿐만 아니라 bcl-2/ bax 비율의 변화가 관여된다는 것이 in vivo에서 확인되었다. p53가 자연형인 경우에도 그 이하 단계의 유전자 발현 양상이 다르게 나타날 수 있으며 이는 세포 독성 요인을 이용한 암 치료시 결과를 예측하는데 있어서 단일 유전자 발현의 평가와 연계되는 복잡성을 시사하고 있다.