RADIATION-INDUCED APOPTOSIS OF TWO NASOPHARANGEAL CARCINOMA CELL LINES

WANG Feng-wei 王凤玮 [1], LIANG Ke 梁克 [2], YIN Wei-bo 殷蔚伯 [2]
SHEN Yu 沈瑜 [2], SHENG Xiu-gui 盛修贵 [2]

[1]*Department of Radiotherapy, Tianjin Cancer Institute & Hospital, Tianjin Medical University, Tianjin 300060, China*
[2]*Cancer Institute & Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100021, China*

Abstract

Objective: To study apoptosis induced by radiation in two nasopharyngeal carcinoma (NPC) cell lines, CNE and CNE-2. Methods: Hoechst 33342 staining, immunohistochemical staining, RT-PCR, DNA dot blotting and Southern blotting were used to identify apoptosis. Results: A single dose of X-irradiation resulted in apoptosis, the apoptotic index (AI) was time- and dose-dependent. Different apoptotic responses existed in the two cell lines. Immunohistochemical staining showed that bcl-2 protein was strongly positive in CNE but negative in CNE-2. However, RT-PCR revealed p53 mRNA in CNE-2 but not in CNE. P53 and bcl-2 genes were both present in the two cell lines as shown by DNA blotting, but the 2.8 kb fragment of the p53 gene was much lower than the 5.6 kb fragment on CNE which was clearly shown in Southern hybridization, suggestive of partial deletion of p53 gene in CNE. Conclusion: Apoptotic response to radiation is different in two NPC cell lines. CNE is more radioresistant than CNE-2. Overexpression of bcl-2 protein and partial deletion of p53 gene may explain their difference in radiosensitivity.

Key words: Tumor cell line, Radiation, Apoptosis, p53 gene, bcl-2, Nasopharyngeal neoplasm.

Apoptosis now has become a hot spot of cancer research because it is regarded that apoptosis has relationship with carcinogenesis, development, treatment and prognosis.[1-3] Many factors can induce apoptosis, including radiation; simultaneously many genes can regulate the development of apoptosis, some of them are negative regulators, others are positive ones. Recently, p53 and bcl-2 genes are studied and thought to be specially important ones in apoptotic regulation. Overexpression of p53 and bcl-2 protein are frequently observed in specimens of NPC, especially p53 protein which is positive in 80 percent NPC patients in the north of China. It is well known that NPC is most common in China and the major treatment modality is radiotherapy. So apoptosis induced by radiation in NPC cell lines are investigated in order to analyze the relationship among apoptosis, radiation dosage and p53 and bcl-2 genes.

Accepted for publication: July 23, 1998
This work was supported by the National Natural Science foundation of China.
Correspondence to: WANG Feng-wei; Department of Radiotherapy, Tianjin Cancer Institute & Hospital, Tianjin Medical University, Huan Hu Xi Road, He Xi District, Tianjin 300060, China; Phone: (0086-22)-23359929 ext. 380; E-mail: tcih@tjlink.tisti.ac.cn

MATERIALS AND METHODS

Cell Lines and Irradiation

CNE and CNE-2 cell lines were derived from two different patients with NPC, and were maintained in DMEM medium supplement with 10% fetal bovine serum (FBS) and 100 μg/ml penicillin and streptomycin. Radiation was given with a 6 MV-X ray accelerator, the dose rate was 2.5 GY/min, SSD was 100 CM and size of port was 10×cm10cm. A 1.5cm-thick wax board was used when the cells were irradiated.

P53 Gene Inducer

Inducer of exons of 5-10 of p53 gene was constructed by Yuanping biological product corporation, the sequence of inducers were listed below:

sense 5'-ACT-TTT-CGA-CAT-AGT-GTG-GTG-

GTG-CCC-TAT-3'
antisense 5'-CCA-AAA-TGG-CAG-GGG-AGG-GA-3'

Fluorescent Staining Assay

Hoechst 33342 (concentration 30 mg/ml) 2 μl was added after cells were harvested with trypsin/EDTA. Cells were observed under a fluoroscope after 10 min cultured in a thermostat at 37°C. Characteristics of apoptotic cells were (1) integrity of cell and nucleus membrane; (2) cells containing one or more condensed masses of nuclear chromatin, usually round or crescent shaped; (3) volume of apoptotic cells shrunk; (4) a cluster of smaller membrane-bound apoptotic bodies.

Three 50 high-power fields were selected randomly for each specimen to quantify the AI, and the result was confirmed by another investigator simultanously.

Immunohistochemical Staining Assay

The method used in our trial referred to Shangrong Ni et al..[4]

P53 mRNA Extraction and RT-PCR

Referred to Shengdong Lu et al.[5]

DNA Southern Blot Analysis

Referred to Shengdong Lu et al.[5]

RESULTS

Dose-and Time-Dependent on Apoptosis in NPC Cell Line after Irradiation

A single dose of 2, 4, 8, 12 and 20 Gy resulted in apoptosis in CNE cell line, apoptotic index (AI: the percentage of apoptotic cells) was quantified in 0, 10, 18, 24, 44 and 48 h after irradiation and results were presented in Figure 1. We could see that AI increased gradually at the beginning and reached a plateau at 44–48 h after irradiation in each single dose group. So 48 h was determined as the observing and quantifying time after irradiation. The occurrence of apoptotic cells was reassured by DNA agarose electrophoresis and flow cytometry assay.

Different Apoptotic Response between CNE and CNE-2

AI induced by radiation in CNE and CNE-2 cell lines were presented in Figure 2. After a single dose of 0, 2, 4, 8, 12, and 20 Gy, the AI was 2%, 10.2%, 17.8%, 21.4%, 24% and 27% respectively in CNE, whereas it was 3.5%, 15%, 20%, 31%, 38% and 48% respectively in CNE-2 cell line. Obviously, there was apparently different apoptotic response between two NPC cell lines. In order to interpret the intrinsic mechanism of this phenomenon, several methods were used. Immunohistochemical staining showed that bcl-2 protein was strongly positive in CNE but negative in CNE-2. However, RT-PCR revealed p53 mRNA in CNE-2 but not in CNE. P53 and bcl-2 genes were both present in the two cell lines as shown by DNA blotting, but the 2.8 kb fragment of the p53 gene was much lower than the 5.6 kb fragment in CNE which was clearly shown in Southern hybridization, suggestive of partial deletion of p53 gene in CNE.

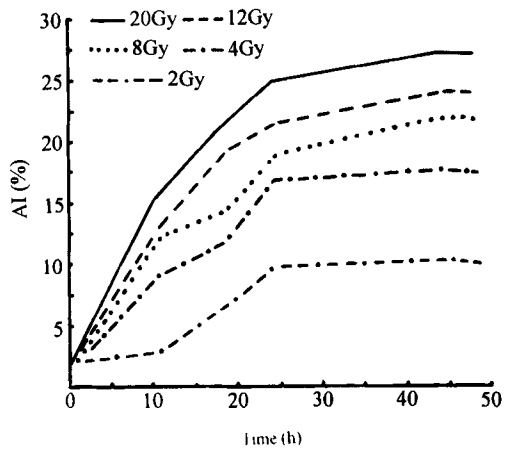

Fig. 1. Dose-and time-dependent on apoptosis in CNE

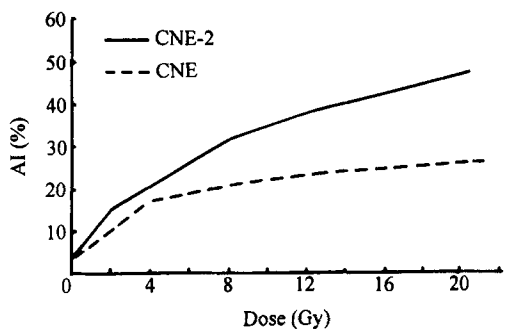

Fig. 2. Apoptotic responses in CNE and CNE-2

DISCUSSION

It is shown that different apoptotic response existed between two NPC cell lines. AI increases abruptly from 0 to 4 Gy in CNE, then increase slowly and nearly reach a plateau at 20 Gy. AI is higher in CNE-2 than that in CNE, and it elevates nearly at the same rate according to the single dose given. So AIs are apparently different in the latter parts of the two

curves; thus, split dose in clinic in two NPC cell lines should not be the same, it should be adjusted by the dose-AI curve.

Ressel et al.[6] thought that this phenomenon reflect the different intrinsic radiosensitivity in tumors and there are no exact mechanisms to explain it. However, we find that bcl-2 protein is strongly positive and there is partial deletion of p53 gene in CNE. Bcl-2 gene is thought to be an oncogene, and its overexpression is frequently observed in many kinds of human carcinoma, such as lymphoma, colon and prostate adenocarcinoma, squamous cell carcinoma of lung, neuroblastoma and NPC etc. Several factors can influence and regulate the level of bcl-2 protein and mRNA, including IL-2, IL-6, TGF-6, LMP-1 protein and retinoid.[7] LMP-1 protein of EB virus derived from NPC patients can promote overexpression of bcl-2 protein, whereas bcl-2 protein can inhibit apoptosis induced by radiation, serum deprivation and high concentration oxygen. So we postulate that in CNE bcl-2 gene is regulated by LMP-1 protein which leads to the decrease of AI. Partial deletion of p53 gene resulted in negative mRNA in CNE leads to the negative expression of p53 protein, which can inhibit the occurrence of apoptosis via p53 gene pathway.[8] Thus we think CNE is more radioresistant than CNE-2, and overexpression of bcl-2 protein and partial deletion of p53 gene may explain their difference in radiosensitivity.

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