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THE STUDY OF ELEMENE OF INDUCTION APOPTOSIS ON ASCITES HEPATOMA CELL LINE Hca-F25/CL-16A3

Zuo Yunfei 左云飞 Zhang Yaozheng 张耀铮
Department of Clinic Biochemistry, Dalian Medical University, 116023

Zhang Hong 张红
Department of Traditional Chinese Medicine, Dalian Medical University, 116027

Objective: To investigate the effect of inducing apoptosis of Elemene on ascites hepatoma cell line Hca-F25/cL-16A3. By using immunhistochemistry and DNA electrophoresis, the mechanism of Elemene antitumor was studied. Results: The results showed that the Elemene can inhibit expression of *bcl*-2 in ascites hepatoma cell line Hca-F25/CL-16A3, and the Elemene also can make DNA fragmentation in this cell line *in vitro* and *in vivo*. Conclusion: The data suggest that Elemene can inhibit the growth of tumor by inducing apoptosis.

Key words: Apoptosis, *bcl*-2, Hca-F25/CL-16A3, Immunohischemistry.

Recent progress in the study of the molecular mechanism of cancer had contributed to our better understanding of antitumor. Under such circumstances, it may be possible to discover the drug and explore their roles in the mechanisms of antitumor. Tumor growth, whether benign or malignant, results from an imbalance of cell production and cell loss. In 1977, Steel calculated that in some malignancies, the rate at which new cells were produced was almost equaled by the rate of spontaneous cell loss.[1] Consequently, the factor which alters tumor cell loss, or increases cell death or exfoliation, could has a profound influence on tumorigenesis. One of the most important mechanisms of tumor cell loss is apoptosis or programmed cell death. Recent work suggests that several genes are involved in mediating programmed cell death.[2] Intriguingly, some of these are already known to be regulators of cell proliferation and differentiation, suggesting that the decision, whether a cell divides or dies involves closely related pathway. The *bcl*-2 is the important mediators of apoptosis. *Bcl*-2, located at chromosome locus 18q, encodes a 26 kilodalton protein which resides in the mitochondrial membrane, endoplasmic reticulum and nuclear envelope.[3,4] When expressed in tumor it prolongs cell survival and rescues them from apoptosis induced by a variety of agents.[5] The death of cells undergoing apoptosis is preceded by chromatin cleavage at the linker regions between nucleosomes by specific endonucleases, which results in extensive fragmentation of the DNA subunits. The DNA fragments can be demonstrated by agarosegel electrophoresis. In this paper, by using immunohistochemistry and DNA electrophoresis. We studied the mechanisms of apoptosis by Elemene induced.

MATERIALS AND METHODS

Agents and Animals

Tumors were obtained from *bcl* B/C pureline mice after 5×10^6 cells were injected in groin. Elemene was injected in mice abdomen of experimental groups and 0.9% NaCl were injected in control groups every day,

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then mice were killed after a week.

Expression of *bcl*-2 Gene

Immunohistochemical staining for *bcl*-2 gene expression was performed by a peroxidase-labeled streptavidinbiotin techniques. 4-Micron paraffin tissue sections were munted on aminoalkysilance-treated glass slides and were deparaffinizied in xylene and alcohol and placed for 15 min, in alcohol-H_2O_2 for blocking endogenous peroxidase. Sections were treated with sheep serum to prevent background staining. The immunohistochemical technique application of diluted *bcl*-2 antibody 1:100 overnight at 4ºC. Slides were rinsed with phosphate buffer saline for 5 min, and in cubated with the biotinylated linked second antibody for 30 min, and then with the labeling reagent, peroxidase conjugated streptavidine for 30 min. After the slides were rinsed, the peroxidase label was demonstrated using diaminobenzidine in the presence of hydrogen peroxide. Section were counterstained with a light hematoxylin stained slides were mounted with a coverslip.

DNA Electrophoresis

For DNA electrophoresis, tumor tissue cut, centrifuged at 200 g for 10 min and dissolved in hypotonic lysing buffer (100 mM NaCl, 10 mM Tris, 1 mM EDTA, 1% SDS, 0.1 mg/ml proteinase K, pH 7.5) at 37ºC for 1 h. RNAse A (100 g/ml) was added to each sample at 37ºC for 1 h. DNA was extracted with phenol plus chloroform plus isopentane, and recovered by centrifugation at 110000 g for 10 min, water phase was obtained, and them ethanol with 10% 3 M NaAc was added in it, after overnight at –20ºC, centrifuged at 11000 g for 10 min. Pellets were aired dried, dissolved in 10 mM Tris-Hcl, 1 mM EDTA (pH 8.3). Electrophoresis was carried out in TBE buffer (2 mM EDTA, 89 mM boric acid, 89 mM Tris, pH 8.3) and DNA visualized by ethidium bromide staining.

RESULTS

DNA Fragmentation Assays (Figure 1, Figure 2)

Agarose gel electrophoresis of DNA extracted from Hca-F25/CL-16A3 cell line, after 3 h incubation using Elemene (0.3 µg/ml). The DNA something like comet of Elemene-treated Hca-F25/CL-16A3 cell line, greatly different that of controls no DNA-comber likely was seen in control group. Agarose gel electrophoresis of DNA extracted from tumor tissue of Hca-F25/CL-16A3 cell line after 7 days in toto using Elemene. The DNA ladder of Elemene-treated greatly different than that of controls (no DNA fragments were seen in control group).

bcl-2 Expression

Over expression of *bcl*-2 protein in the tumor tissue of Hca-F25/CL-16A3 cell line immunohistochemical staining for *bcl*-2 revealed cytoplasmic staining, no nuclear staining was seen. Among the two groups the Elemene-groups were less weakly positive (56%) than that of control group (96%) (Figure 3, Figure 4).

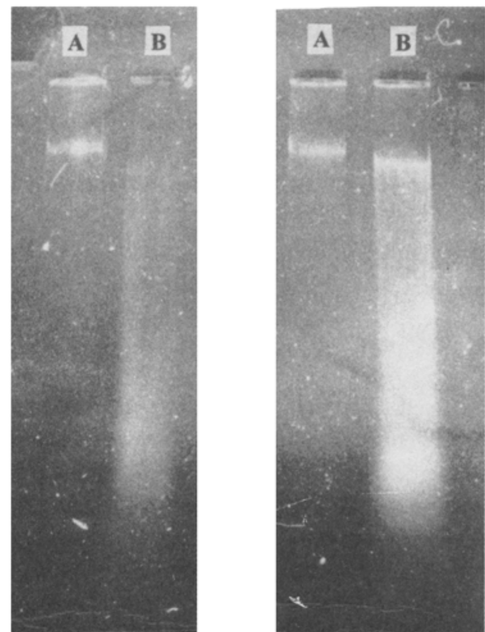

Fig. 1. A: Control group; B: The DNA something like comet of Elemene-treated.

Fig. 2. A: Control group; B: The DNA ladder of Elemene-treated.

DISCUSSION

Development of cancer is the result of the accumulation effects of multiple genes, many of which include oncogenes and tumor suppressor genes. In the

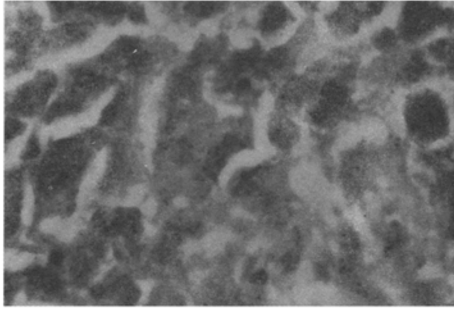

Fig. 3. Control groups were positive (96%).

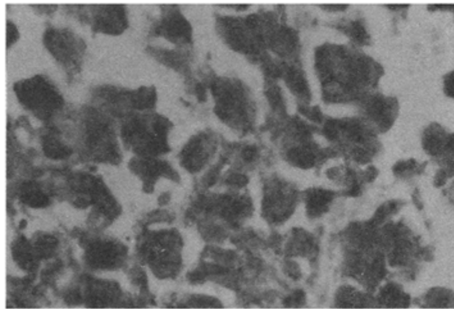

Fig. 4. The Elemene-groups were less weakly positive (56%).

normal cell these genes regulate the cell proliferation and differentiation. More recently, however, attention has been focused on another oncogenes which promote neoplastic transformation by extending cell survival. These genes which play a role in the regulation of cell death by apoptosis. Apoptosis is a common mode of cell loss in tumors. Where it occurs both spontanrously and as a result of cytotoxic therapy. In some cancers the rate of spontaneous cell loss even approaches to that of cell production. Consequently, genetic changes which protect a cell against programmed cell death may have a significant effect not only on the rate of a tumor growth but also on the ability of tumor cells to survive in the hostile environment.[6] In this article, Elemene was shown to have antitumor activity in ascites hepatoma cell line Hca-F25/CL-16A3. The apoptosis of tumor cells was confirmed by the assay of DNA ladder formation on gel electrophoresis and expression of *bcl*-2 protein in the tumor tissue. These results indicate that inducing of apoptosis is one of the mechanisms for antitumor activity of Elemene.

REFERENCES

1. Steel GG. Growth kinetics of tumors. London: Oxford University Press. 1997.
2. Kerr JFR, Winterford CM, Harmon BV. Apoptosis. Its significance in cancer and cancer therapy. Cancer 1994; 73:2013.
3. Tsujimoto Y, Croce CM. Analysis of the structure transcripts and protein products of *bcl*-2, the gene involved in human follicular lymphoma. Proc Natl Acad Sci USA 1986; 83:5214.
4. Akao Y, Otsuki Y, Kataoka S, et al. Multiple subcellular localization of *bcl*-2: detection in nuclear outer membrane, endoplasmic reticulum and mitochondrial membranes. Cancer Res 1994; 54:2468.
5. Vaux DL, Cory S, Adams JM. *Bcl*-2 gene promotes haemopoeitic cell survival and cooperates with c-*myc* to immortalize pre-B-cells. Natures 1988; 335:440.
6. Nicoletti G, Migliorati G, Pagliacci MC, et al. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. Immunological Methods 1991; 139:271.