Study of apoptosis and c-Jun expression in mouse cancer model systems

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Cancer is now recognized to be a disease involving both uncontrolled proliferation and a lack of normal cell death, also known as apoptosis. Cell culture and mouse models of cancer help us to understand this disease as a complex of genetic and biochemical signaling pathways that modulate the cell cycle and death machinery. The c-Jun protein is the product of the immediate-early gene, which has been implicated in the control of cell proliferation and differentiation and more recently in the regulation of apoptosis. We have investigated the role of c-Jun in drug-induced apoptosis in transplantable mouse hepatoma MH-22A and Ehrlich ascites cells. Both primary cell lines were started from mouse tumours. The cells were treated with chemotherapeutic agents – cisplatin, taxol, etoposide and colchicin. All these anticancer drugs have different intracellular molecular targets. After appropriate treatments c-Jun expression and apoptotic response was examined. The study of anticancer drug-induced tumor cell death showed that MH-22A cells were rather resistant to taxol and colchicin. Much more these cells were sensitive to the treatment with cisplatin and etoposide. Ehrlich ascites cells were more resistant to cisplatin than MH-22A cells. Apoptotic response of Ehrlich cells to colchicin tends to be rather similar to that of hepatoma cells. The data obtained indicated a constitutive high level of Jun protein in the MH-22A studied cells. On the contrary, the c-Jun level in Ehrlich cells was appreciable but not high. In MH-22A cells expression of c-Jun did not change greatly after chemotherapeutic treatments. However, treatments with cisplatin and colchicin induced c-Jun synthesis in Ehrlich cells. The results suggest that the drug-induced death process in the mice tumor cells studied may be related to the expression of c-Jun, but the signaling pathways in these cells depend on the kind of tumor.

Key words: cancerogenesis, apoptosis, c-Jun protein, chemotherapy

INTRODUCTION

Apoptosis is a form of regulated cell death to eliminate superfluous cells from the organism. It plays an indispensable role in embryogenesis, in adult tissue homeostasis, but can also contribute to the pathogenesis of a number of human diseases when deregulated. Postgenome drug discovery gave an opportunity to identify the multiple genes that control predisposition of complex diseases, including cancer, as well as individual variation in responses to the drugs that are used in therapy. Many of genetic changes selected during the development of cancer cells occur in the basic signalling pathways that regulate apoptosis. In that way, signalling pathways in apoptosis represent potential targets for cancer therapy. Understanding of the way in which cells, both normal and malignant, die after drug-induced damage would lead to improvements in chemotherapy [1, 2].

A number of proteins with properties of transcription factors that can trigger apoptotic cell death have been identified. One of them is the product of protooncogene *c-jun* [3, 4]. It has been implicated in the control of cell proliferation and differentiation and more recently in the regulation of apoptosis [5, 6]. In order to study the regulatory mechanisms of cell death we investigated expression of c-Jun and apoptosis in drug-induced transplantable mouse hepatoma MH-22A and Ehrlich ascites cells.

Tissue culture and mouse models are widely used as model systems in the studies of tumorigenesis. The ability to control environmental exposure that leads to tumorigenesis or to apoptosis allows a fo-

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cus on molecular components of disease that is not possible with human populations. The parallels, both biochemical and genetic, between the processes of cancer development in humans and mice are particularly striking, leading many laboratories to exploit the mouse as a model organism for the study of this complex disease [7]. The knowledge of interspecies differences in the functioning of signalling pathways in mouse and human cells might well be exploited in the future design of mouse models for human cancer treatment and in the development of anticancer drugs.

MATERIALS AND METHODS

Cell culture

Primary MH-22A cell culture was started from transplantable hepatoma of CBA strain mice. The cells were carried out in modified Eagle medium supplemented with 10% of foetal calf serum.

Ehrlich cells from ascites of Balb/c mice were harvested in a standard incubation medium. They were washed and propagated in modified Eagle medium with foetal calf serum equally well as a permanent suspension culture.

Treatment of cells with chemotherapeutic agents

Both kinds of cells were treated with chemotherapeutic agents for various periods of time. All chemicals used for apoptosis treatments were purchased from Sigma-Aldrich (St. Louis, MO). Taxol was prepared as 10 mg/ml stock solution in DMSO. Both cisplatin (cis-platinum (II) diammine dichloride) and colchicin were dissolved in saline as 10 mg/ml stock solutions. Etoposide was prepared as 50 mM stock solution in DMSO. Cells were treated with a final concentration 10 μg/ml of cisplatin 6 μM of taxol, 10 μg/ml of colchicin and 50 μM of etoposide in the complete medium. C-Jun expression was registered after 1, 2 (or 5) and 20 hours of treatment. Apoptotic response was examined after 72 hours.

Apoptotic cell death analysis

Viability of cell population was examined using the acridine orange/ethidium bromide staining technique [8]. Using this method, both normal and apoptotic nuclei in viable cells displayed a bright green fluorescence, whereas normal or apoptotic nuclei in nonviable cells stained bright orange. Chromatin-free cells exhibit a very weak green-orange staining.

Western blot analysis of c-Jun expression

After appropriate treatments, the cells were collected, washed in ice-cold PBS and lysed with lysis buffer [9]. Cell lysates were resolved on 10% SDS-PAGE, transferred to polyvinylidene difluoride membranes and blocked in Blotto. Membranes were probed with rabbit monoclonal anti-c-Jun primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies (New England BioLabs). Proteins were detected using the enhanced chemiluminescence (ECL) method according to manufacturer's instructions (Amersham Pharmacia Biotech).

RESULTS AND DISCUSSION

A number of reports have suggested that c-Jun may induce cell proliferation [5, 10]. However, strong and prolonged induction of *c-jun* has also been reported in response to a variety of stress-inducing treatments that can trigger apoptosis [6, 11]. In searching for the factors that may be related to the apoptotic response to anticancer drugs in mouse hepatoma MH-22A and Ehrlich ascites cells, we just investigated the expression of c-Jun protein. Both these primary cell lines were initiated from mouse tumors. The cells *in vitro* were treated with chemotherapeutic drugs, such as cisplatin, taxol, colchicin and etoposide. All these anticancer drugs have different intracellular molecular targets: cisplatin reacts with DNA inducing inter-, intrastrand cross-links, etoposide is topoisomerase I inhibitor causing single strand DNA breaks, whereas taxol and colchicin are microtubule-directed agents [12–15].

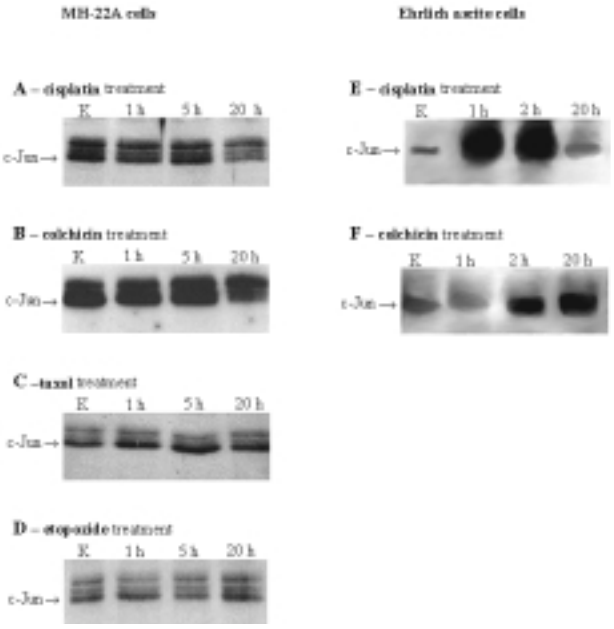

Fig. 1. Expression of c-Jun protein in MH-22A and Ehrlich ascite cells in control (**K**) cells and after **1**, **2** (or **5**) and **20** h treatment with cisplatin – **A** and **E**; with colchicin – **B** and **F**; taxol - **C**; and etoposide – **D**

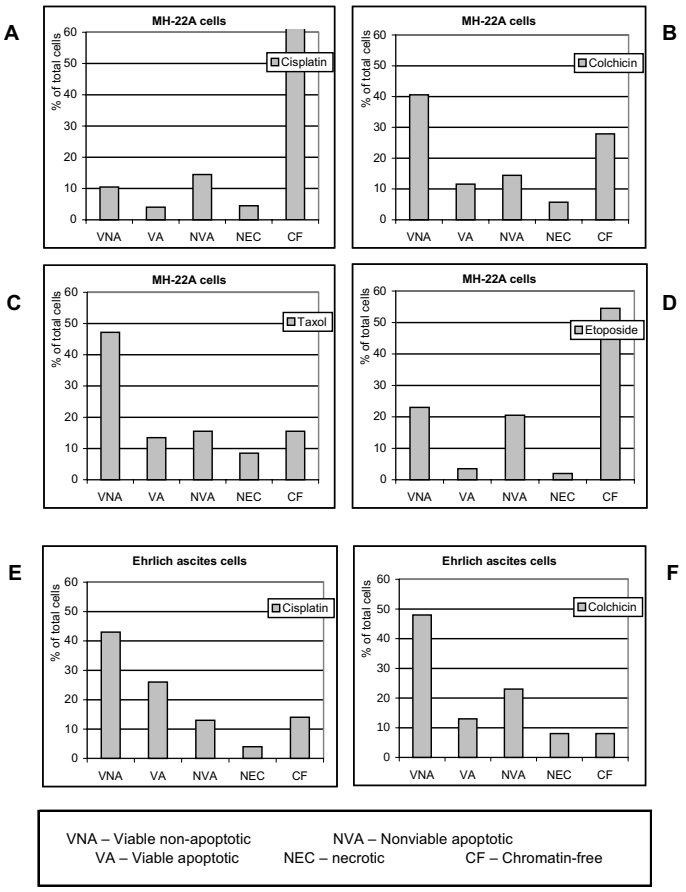

Fig. 2. Evolution in the percentage of viable-nonapoptotic, viable apoptotic, non-viable apoptotic, necrotic, and chromatin-free MH-22A and Ehrlich ascite cells after 72 hours of treatment with cisplatin – **A** and **E**; with colchicin – **B** and **F**; taxol – **C**; and etoposide – **D**

The data obtained indicate a constitutive high-level of Jun protein in MH-22A cells. After chemotherapeutic treatments c-Jun expression did not change greatly. A slight decrease of c-Jun level was detected after 20 h of treatment with cisplatin and colchicin (Fig. 1 A and B). In both above-mentioned cases as well as at 20 h after treatment with etoposide (Fig. 1 D) a slight increase of the phosphorylated form of c-Jun was registered. Taxol did not influence the c-Jun level in the mouse hepatoma MH-22A cells studied (Fig. 1 C) as well as in human KB-3 carcinoma cells [16].

The study of apoptotic response of these cells to anticancer drugs showed that MH-22A cells were rather resistant to the taxol and colchicin treatment. Data presented in Fig. 2 (C and B) show that only about 25% of apoptotic cells (both viable and non-viable) were determined after treatment with these anticancer drugs. Considerable response was detected after cisplatin and etoposide treatment of hepatoma cells (Fig. 2 A and D). Large amounts of chromatin-free and non-viable apoptotic cells demonstrated a possible early effect of these drugs in MH-22A culture.

The results of apoptotic response and c-Jun expression in mouse hepatoma MH-22A cells were compared with the results obtained in the study of Ehrlich mouse ascites cells. The comparison showed that c-Jun expression in Ehrlich cells was appreciable but not high. We found that c-Jun level in MH-22A cells did not change greatly after treatments. However, in Ehrlich cells treatment with cisplatin and colchicin enhanced c-Jun synthesis markedly. Data presented in Fig. 1 (E and D) show that cisplatin induced c-Jun expression as soon as after 1 h of exposition. At that time also the phosphorylated form of protein was registered. However, the induction was not long-term and about 20 h following the treatment the protein level was close to the baseline. A considerable induction of c-Jun after colchicin treatment was observed from the 2nd hour. Apoptotic response of Ehrlich ascites cells showed that they were more resistant to cisplatin than MH-22A cells. The sensitivity of ascites cells to colchicin was similar to that of hepatoma cells.

Studies on the c-Jun expression in F-MEL and NIH 3T3 cells indicate that this protein at a level above the threshold induced apoptosis [1, 4]. The apoptotic response of cells to cisplatin in our study suggested that a high level of protein c-Jun contributed to apoptosis, but cell response to colchicin gives rise to doubts. Preliminary results of the application of antisense c-Jun method to MH-22A cells are under way. They show that blocked synthesis of Jun contributes to cell proliferation and stops apoptosis.

Yet c-Jun is located at the end of signal cascades that include important oncogenes active in human tumours. This position in cellular signalling makes c-Jun a participant in numerous and diverse mechanisms of oncogenesis [17]. C-Jun activity may therefore be a decisive factor in many tumours. Regulating this activity appears a worthy and promising goal. Mouse models of tumour help us to understand cancerogenesis as a complex of biochemical processes involved in the pathways that affect individual cancer susceptibility.

Received 14 October 2002

ACKNOWLEDGEMENTS

This work was supported by the Lithuanian State Science and Studies Foundation
(Grants Nos K-024 and G-046).

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APOPTOZĖS IR BALTYMO C-JUN EKSPRESIJOS TYRIMAS MODELINĖSE PELIŲ NAVIKŲ SISTEMOSE

S a n t r a u k a

Šiame darbe tirta baltymo c-Jun raiška ir jo vaidmuo chemoterapiniais preparatais paveiktose pelės hepatomos MH-22A ir Erlicho ascito ląstelėse. Abiejų navikų ląstelės buvo išskirtos iš audinio ir auginamos *in vitro*. Gavus pirminę liniją, jos buvo paveiktos priešvėžiniais preparatais – cisplatina (10 μg/ml), taksoliu (6 μM), etopozidu (50 μM) ar kolchicinu (10 μg/ml). Nustatėme, kad pelės hepatomos ląstelėse yra konstitutyviai aukšta baltymo c-Jun raiška. Erlicho ascito ląstelėse šio baltymo rasta gerokai mažiau. MH-22A ląstelėse chemoterapinių medžiagų veikimas beveik neturėjo įtakos c-Jun ekspresijai. Tuo tarpu Erlicho ascito ląstelėse cisplatina ir kolchicinas indukavo c-Jun sintezę. Palyginus c-Jun sintezės ir apoptozės tyrimus, manome, kad priešvėžiniais preparatais indukuotas ląstelių žūties procesas tirtose pelių navikų ląstelėse galėtų būti siejamas su baltymo c-Jun kiekiu, tačiau signaliniai keliai šiose ląstelėse yra skirtingi ir tikriausiai priklauso nuo naviko rūšies.