Chinese Journal of Cancer Research 10(4): 235-238, 1998

EFFECT OF UP-REGULATION OF S-ADOMET SYNTHETASE ON TAXOL-INDUCED APOPTOSIS IN HUMAN BREAST CANCER CELLS

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Objective: To investigate the gene regulation of taxol-induced apoptosis.Methods: Northern blot hybridization, enzyme activity assay of S-AdoMet synthetase and flow cytometry were performed in the investigation of expression in the mRNA level and biological action of S-AdoMet synthetase in taxol-induced apoptosis in human breast cancer cell line (BCap 37). Results: Up-regulation of S-AdoMet synthetase expression was resulted by taxol treatment and the expression peaked at 48 hours.Moreover,the up-regulation of S-AdoMet synthetase was associated with cytotoxicity of anti-microtubule agents including taxol and colchicine. Inhibition rate of S-AdoMet synthetase activity by 1% DMSO was 34% in taxol-treated cells and 14% in taxol-untreated cells compared to control groups, respectively. Posttreatment with 1% DMSO following pretreatment with individual antitumor agent for 3 hrs promoted apoptotic cell death of taxol-,colchicine-,and adriamycin-treated Bcap37 cells. Conclusion : The induction of apoptosis enhanced by post-treatment with DMSO in taxol-treated cells is probably linked to its inhibition on enzyme activity of S-AdoMet synthetase ,suggesting that the increased expression of S-AdoMet synthetase possibly plays an important role in protecting cells from DNA fragmentation in taxol-induced apoptosis.

Key words: Breast neoplasm, Taxol, S-AdoMet synthetase, Apoptosis

We have isolated twelve cDNA clones associated with taxol-induced apoptosis using mRNA differential display in the mRNA level, in which clone C3P3 had 99% homology with human S-Adenosylmethione(S-AdoMet) synthetase by searching against Genbank and EMBL databases. Furthermore, in taxol-induced apoptosis, both the up-regulation of S-AdoMet synthetase in the mRNA level and increased activity of S-AdoMet synthetase had been confirmed [1,2]. In the present study, we aimed to investigate the expression specificity and biological action of S-AdoMet synthetase in taxol-induced apoptosis applying Northern blot hybridization and dimethyl sulfoxide (DMSO) as inhibitor of S-AdoMet synthetase.

MATERIALS AND METHODS

Cell Culture and Drugs

Human breast cancer cell line(BCap 37) was grown in same condition as previously shown.[1] Taxol was purchased from Calbiochem(USA). Colchicine、cis-platinum、camptothecin、etoposide (or VP-16)、adriamycin and cycloheximide were purchased from Sigma Chemical Co(USA). Stock solutions were made by dissolving agents in 100% DMSO, except for adriamycin and cycloheximide which were dissolved

Accepted July 26, 1998
This work was supported by the National High Biotechnology Foundation of China and a grant from the Natural Science Foundation of Zhejiang Province, China.

in dH_2O. The working concentrations of taxol、colchicine 、cis-platinum 、camptothecin 、etoposide、adriamycin and cycloheximide used were 100nmol/L、50nmol/L、10 μmol/L、10μmol/L、10μg/ml、10μg/ml and 5μg/ml, respectively.

In experiments involving DMSO post-treatment, cells were firstly treated with the anti-cancer agents indicated for 3 hours,then washed twice with fresh medium, and incubated in fresh medium containing 1% DMSO for an additional 72 hours.

Flow Cytometry Analysis

Cell samples were prepared and stained with propidium iodide (PI) for flow cytometric analysis according to the method shown by Chen et al. [2] In the present experiment, a $1x10^6$ of cells treated with anti-cancer agents followed by DMSO treatment as above described were harvested by trypsinization and washed twice in cold PBS.Cell cycle distribution was determined with an Epics Elite instrument (Coulter Corp,USA)with an argon laser set to excite at 488 nm. The results were analyzed by Elite 4.0 and DNA multicycle software(Phoenix Flow System, San Diego,USA).

Measurement of S-AdoMet synthetase activity

BCap 37 cells treated with taxol for 3 hours followed 1% DMSO treatment for 24 hours, taxol alone and DMSO alone as well as control cells were harvested and then washed twice with PBS. From each group, $4x10^6$ cells were suspended in 50 mmol/l HEPES/KOH buffer and sonicated with a w380 sonicator.After removal of debris by centrifugation for 10 min at 10,000xg, aliquots were removed for the S-AdoMet synthetase enzyme activity assay by the method of Markham et al . [3]

Northern Blot Hybridization

10 μg of total RNA , extracted from the treated cells by the acid guanidine-thiocyanate method, was seperated by electrophoresis on a 5.4% formaldehyde/1.0% agarose gel in 1xMOPS buffer.The seperated RNA was saturated in a 10xSSC solution and then transferred to a nylon membrane (Stratagene,USA) in a 20xSSC solution with a Posiblot Pressurer (Stratagene,USA) at 60 mmHg for 2 hours.RNA on the membrane was cross-linked by an ultraviolet cross-linker(Stratagene). Using clone C3P3 cDNA as template,we prepared a P^{32}-labeled RNA ribo-probe with a commercial RNA transcription kit (Stratagene). Prehybridization of the RNA was carried out in 7%SDS, 0.25 M $NaHPO_4$, 1 nM EDTA and 100μg/ml yeast tRNA at 65℃ for 2 hours, then about $1x10^7$ cpm of the total radioactivity of RNA ribo-probe was added to the pre-hybridization buffer for hybridization reaction at 65 ℃ overnight. The hybridized membrane was wash twice for 15 minutes each in 2xSSC/0.1%SDS buffer at 65℃ , followed by two washes of 0.2xSSC/0.1%SDS buffer , each for 15 minutes at 65℃. The blot was exposed to the Kodak xomat AR film with intensifying screen at -70℃ for an appropriate time.

RESULTS

Altered Expression of clone C3P3 in taxol-induced apoptosis

Taxol treatment induced BCap 37 cells to gradually increase transcription at the mRNA level of S-AdoMet synthetase gene.At 48 hours, the expression of S-AdoMet synthetase reached a peak.Then,the enzyme expression evidently decreased at 72 hours,even lower than that of control cells shown in Figure 1.

Expression Differences of S-AdoMet synthetase caused by other anti-cancer agents

To determine whether taxol was unique in inducing increased transcription of S-AdoMet synthetase, we examined the effect of other anti-cancer agents on its expression at the mRNA level by Northern blot same as above. Only taxol and colchicine enhanced the expression of S-AdoMet synthetase, whereas cis-platinum, camptothecin, etoposide,adriamycin and cycloheximide decreased its expression when compared with control level shown in Figure 2.

Effect of DMSO on S-AdoMet synthetase enzyme activity

To understand the relationship between an increased expression of S-AdoMet synthetase and induction of apoptosis by taxol, we studied the effects

of DMSO on the enzyme activity in BCap37 cells. A low concentration (1%) of DMSO treatment for 24 hours reduced 14% of its enzyme activity when compared with the control. Moreover, 1% DMSO post-treatment decreased its enzyme activity by 34% when compared with taxol pre-treatment shown in figure 3.

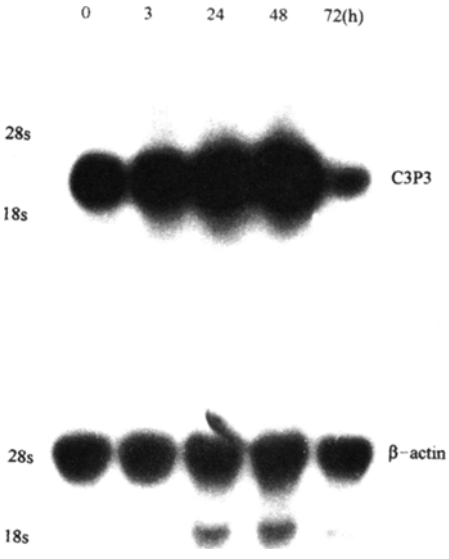


Fig.1. mRNA expression level of clone C3P3 at different times of taxol-treated Bcap cells..

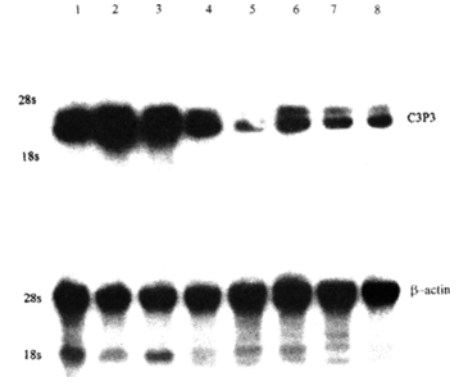
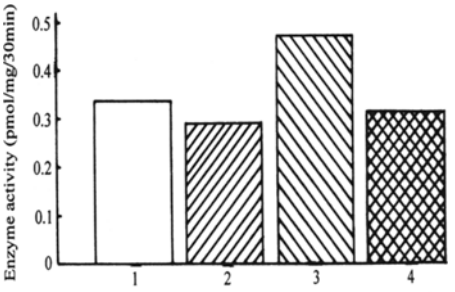


Fig. 2. Expression differences of clone C3P3 in BCap37 cells treated with various agents for 24 hours.

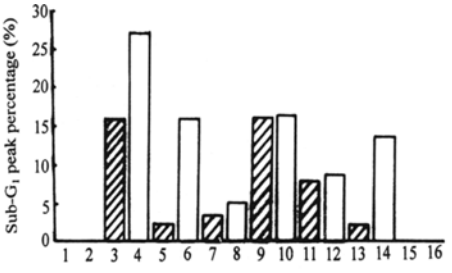
Promoting Effect of DMSO Post-treatment on Taxol-induced Apoptosis

Flow cytometry analysis showed that DMSO treatment at 1% concentration by itself did not alter the cell cycle distribution of BCap37 cells. DMSO treatment after 3 hours of exposure to anti-cancer agents only increased the percentage of Sub-G1 peak (apoptotic peak) in taxol-,colchicine- and adriamycin-treated cells.DMSO treatment did not affect apoptosis processes induced by other anti-cancer agents seen in figure 4.



1. control; 2. DMSO; 3. Taxol;
4. DMSO posttereatment for 24 hrs.

Fig. 3. Inhibition of DMSO posttreatment on enzyme activity of S-AdoMet synthetase of BCap 37 cells induced by taxol



1. control; 2. DMSO; 3. Taxol; 5. Colchicine; 7. cis-platinum; 9. Camptothecin; 11. Etoposide; 13. adriamycin; 15. Cycloheximide; 4,6,8,10,12,14 and 16 for DMSO posttreatment for 72 hrs of 3-15 groups in dicated, respectively.

Fig. 4. Effect of DMSO posttreatment on DNA content changes of BCap 37 cells previously treated with various drugs.

DISCUSSION

In this study, we found that apoptotic cell death induced by taxol may involve the up-regulation at

mRNA level of S-AdoMet synthetase. By 3 hours after taxol treatment of BCap 37 cells, an increased expression of S-AdoMet synthetase happened, which peaked at 48 hours. An increased expression of S-AdoMet synthetase at the mRNA level correlated with an increase in S-AdoMet synthetase enzyme activity of BCap 37 cells. Compared with the time course of the cell cycle block by flow cytometry and DNA fragmentation by electrophoresis in taxol-induced apoptosis,[2] induction of S-AdoMet synthetase expression occurred before the arrest of the cell cycle and DNA fragmenting, suggesting that an increased expression of S-AdoMet synthetase is associated with taxol-induced apoptotic cell death in human breast cancer cells.

To investigate the specificity of up-regulation of S-AdoMet synthetase in taxol-induced apoptosis and its possible function in anti-cancer therapy, we analyzed the expression of S-AdoMet synthetase in apoptosis caused by different kind of anti-cancer agents. We found that up-regulation of S-AdoMet synthetase at the mRNA level occurred in taxol- and colchicine-treated cells, while cis-platinum, camptothecin, etoposide, adriamycin and cycloheximide had no effect, or inhibition on its enzyme expression. This indicated that up-regulation of S-AdoMet synthetase in BCap 37 cell line is not a common event in any anti-cancer agent treatment, but relatively specific one to anti-microtubule drug treatment, because both taxol and colchicine inhibited the formation of the mitotic spindle and blocked cell division at mitosis , but which had different mechanism against dynamic equilibrium of microtubule.[4,5]

S-AdoMet synthetase(ATP: L-methionine S-Adenosyltransferase, EC2、5、1、6) is a key enzyme catalysing the synthesis of S-AdoMet from ATP and L-methionine in the presence of Mg^{2+} and K^{+}. This enzyme and its products , S-AdoMet, have been studied in bacteria, yeast, plants and animal. S-AdoMet acts as the principal donor of methyl group for most transmethylation reaction. Importantly, the transmethylation reaction involves the methylation of DNA, RNA, protein, lipids and numerous small molecules.[6] The biological role of increased expression of S-AdoMet synthetase in taxol-induced apoptosis is unclear. On the base of our previous finding, which BCap 37 cells exposed to taxol(100nmol/L)for 0.5 hours and more underwent apoptosis process without drug concentration maintenance,[2] we designed the DMSO post-treatment experiment to clarify the influence of decreased enzyme activity of S-AdoMet synthetase on taxol-induced apoptosis.The results demonstrated that DMSO post-treatment promoted taxol-,colchicine- and adriamycin-induced apoptotic cell death, whereas it had no effect on apoptotic pathway induced by cis-platinum, camptothecin, etoposide and cycloheximide. Thus, these results suggested that the promoting effect of DMSO post-treatment on taxol-induced apoptosis be probably associated with the inhibition of enzyme activity of S-AdoMet synthetase. In other words, the increased expression of S-AdoMet synthetase might play an important role in protecting cells from DNA fragmenting in taxol-induced apoptosis.

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