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Arsenic trioxide regulates gastric cancer cell apoptosis by mediating cAMP

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Abstract. – OBJECTIVE: Gastric cancer is a common digestive tract tumor in clinic with increasing incidence. It is suggested that arsenic trioxide (As_2O_3) has an inhibitory effect on many kinds of digestive system tumors. This study evaluated the impact of As_2O_3 on the apoptosis of gastric cancer BGC-823 cells, and analyzed its relationship with cyclic adenosine monophosphate (cAMP).

MATERIALS AND METHODS: Gastric cancer cell BGC-823 was intervened by different concentrations of As_2O_3 at 4 ng/ml, 8 ng/ml, a d 16 ng/ml, respectively. BGC-823 cell apopto evaluated by TUNEL assay. Cell cycle wa mined by flow cytometry. cAMP and prot i- nase C (PKC) was detected by radioimmu say. Apoptosis-related protein levels were te by Western blot.

RESULTS: Compared wit l contro As2O3 significantly incre 823 cell apoptosis, blocked cell cy G0/G ase, elevated cAMP and Bax le s well reg- ulated PKC, Bcl-2 and in ex 0.05).

CONCLUSIONS: O_3 ind GC-823 cell apoptosis throu up-regula the cAMP level and the d of the PKC le

Key Words:
As_2O_3, C tric cancer, Ap cAMP, PKC.

Intr ction

limit ration is characteristic of ca cells. E pt for number increase, they ar featured as apoptosis restriction[1,2]. Ar- also known as As_2O_3, is previously d in the treatment of acute myeloid leukemia. recent study[3] revealed that As_2O_3 has the in- ry effect on digestive system neoplasm by in ng cell apoptosis. It is found that the process o ptosis is reg ted by cytokines signa athways, such as Ca^{2+}, cyclic adenosine mo phate (cAMP), protein C (PKC), and ase signaling pathw . The cAMP is an important second messger involved i cell proliferation, division, ptosis, and ot ife activities[6]. PKC is a Ca^{2+} phospholipid pendent protein kinase that ca ulate ce optosis process. PKC elevation s cell apoptosis, while PKC downregu ation increases cell apoptosis rate[7]. his study selected gastric cancer BGC-823 cell ened it with different concentrations of As_2O_3 analyze the effect of As_2O_3 on BGC-823 cell apoptosis.

Materials and Methods

Experimental Cells

Gastric cancer cell line BGC-823 was provided by Xinjiang Medical University Culture Center (Urumqi, Xinjiang, China).

Reagents and Instruments

As_2O_3 was from ProSpec (ProSpec, East Brunswick, NJ, USA). RPMI-1640, penicillin, and streptomycin were provided by Gibco (Thermo Fisher Scientific, Waltham, MA, USA). The cAMP kit and PKC kit were got from Gibco BRL (Thermo Fisher Scientific, Waltham, MA, USA). The centrifuge was from Beckman (Beckman Coulter, Brea, CA, USA).

Experimental Methods

Conventional Cell Culture

BGC-823 cells were cultured in RPMI-1640 medium and maintained in 37°C and 5% CO_2.

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As_2O_3 Intervention

BGC-823 cells in logarithmic phase were seeded in a plate and treated with different concentrations of As_2O_3 at 4 ng/ml, 8 ng/ml, and 16 ng/ml, respectively.

TUNEL Assay

After treated by As_2O_3, BGC-823 cells were washed with PBS and added with TdT enzyme (Merck, Temecula, CA, USA). After added by the anti-digoxin antibody (Abcam, Cambridge, MA, USA), the cells were developed by DAB.

Cell Cycle Detection

BGC-823 cells in logarithmic phase were centrifuged and fixed at 4°C overnight. After treated by PI avoid of light for 30 min, the cells were detected by flow cytometry (BD, San Jose, CA, USA).

Radio Immunoassay

The cells were digested and treated by RIPA (Merck, Temecula, CA, USA) at 0°C for 30 min. After centrifugation, the supernatant was added with 3H-cAMP and 32P-substrate (M... Temecula, CA, USA). After centrifuga... supernatant was treated with scintillation s...n to test radioactivity according to the manual.

Western Blot

Total protein was extracted ... cells and... separated by SDS-PAGE. ... blo...l at RT, the membrane was incuba...n prima...ntibody (1:200, -actin 1:500) ... cond... (1:2000) (Abcam, C...brid... USA) at RT for 1 h in sequenc... ter deve... by buffer A and B, the memb... was read to ... the relative protein e...

Statistical Analysis

SPSS ... software (IBM ... onk, NY, USA) was a... ed for data analysis. The data was presen... as mea... standard deviation. Enumerati... a w... mpare... by χ^2-test, while measure... was c... red by *t*-test. $p < 0.05$ ... con... d as s... ical significance.

Results

As_2O_3 Induced BGC-823 Cell Apoptosis

After treated by As_2O_3, BGC-823 cell apoptos... evaluated by TUNEL assay. Light gray sta...g was observed in BGC-823 cells of the control group, while deep brown staining was shown in that of the experimental gr... apoptosis increased in the experim... group compared with that in the control ...up, which was in a dose dependence manner following the addition of As_2O_3 ($p < 0.05$) (Figure ... 2).

As_2O_3 Blocked BGC-8... Cell Cycle

Different concentrati... of As_2O... were app... in the treatment of BG... 23 cel... 48 h. Flow cytometry was perfo... t... ell cyc... With the elevating dos... of As... amou... cells in G0/G1 phas... dually in... d, ... that in S and G2/M... s declined (...) (Figures 3 and 4).

As_2O_3 Affected cA... d PKC Levels in BG... Cells

...MP and PKC levels in BGC-823 cells were ...d by radioim...unoassay. The result demon...ted that As_2O... arkedly upregulated cAMP ... while redu... PKC level in BGC-823 in a d... pendent ...ner ($p < 0.05$) (Figure 5).

As_2O_3 A...ected Bcl-2, Bax, and Survivin Protein Expressions in BGC-823 Cells

...n blot was performed to determine Bcl-Ba... nd Survivin protein expression in BGC-823 cells after the treatment of As_2O_3. It was found that levels of Bcl-2 and Survivin declined, whereas Bax expression enhanced in the experimental group following the increasing level of As_2O_3 concentration (Table I, Figure 6).

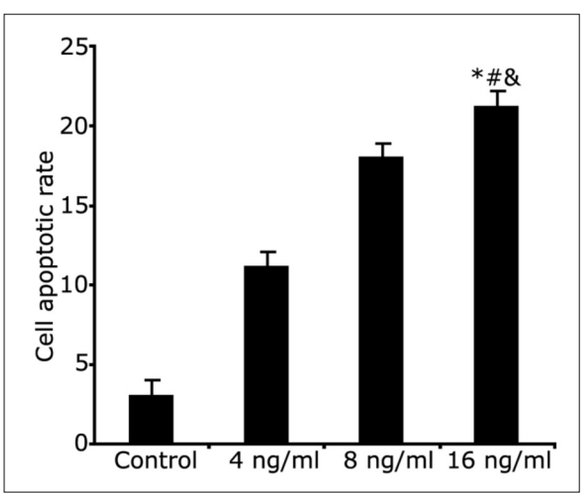

Figure 1. BGC-823 cell apoptosis analysis. $^{*}p < 0.05$, compared with normal control. $^{\#}p < 0.05$, compared with 4 ng/ml group. $^{\&}p < 0.05$, compared with 8 ng/ml group.

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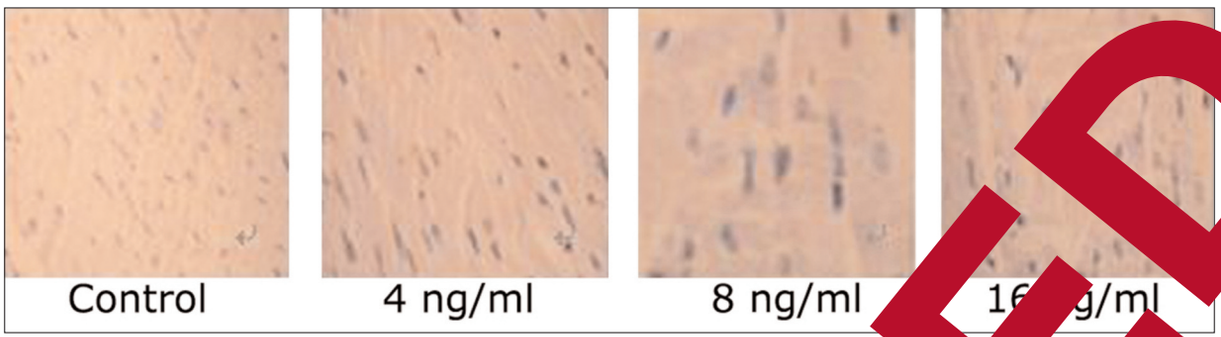

Figure 2. As_2O_3 induced BGC-823 cell apoptosi

Discussion

The formation of the malignant tumor is caused by malignant tumor cell unlimited proliferation, leading to an imbalance between cell survival and death. Cell number unlimited increase eventually forms the malignant tumor[8,9]. It was repo that inhibited DNA and RNA synthesis process, res in chromosome mutati this study, g cancer BGC-823 c ere treated with diffe nt concentrations of O_3 to analyze it mechanism on apoptosis. UNEL assay wed that the cells in the ex- ental group sented deep brown staining u micros The cell apoptosis rate signific d in experimental group compared wi ontrol, and the rate was gradually regulated following the rise of As_2O_3 concen- 's suggested that As_2O_3 can induce gas- er BGC-823 cell apoptosis. It was reported that low concentration of As_2O_3 induced cancer cell apoptosis to suppress tumor growth[11]. Our result also was consistent with previous finding that As_2O_3caused thiol enzyme inactivation, block tumor cell cycle, inhibit tumor cell proliferation, and further induce tumor cell differentiation and apoptosis[12].

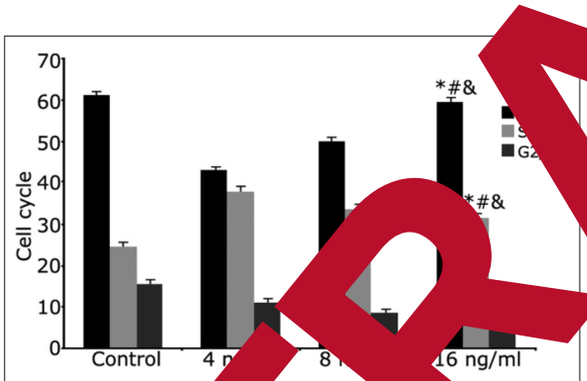

Figure 3. BGC-8 cycle analysis. 05, compared with nor $^{\#}p < 0.05$, com ed with 4 ng/ml group. $^{\&}p$ 05, co with 8 ng/ml group.

Cell cycle analysis revealed that cell rate in G0/G1 phase markedly increased, while in S and G2/M phases declined in BGC-823 cells treated by As_2O_3 in a dose-dependent manner. It

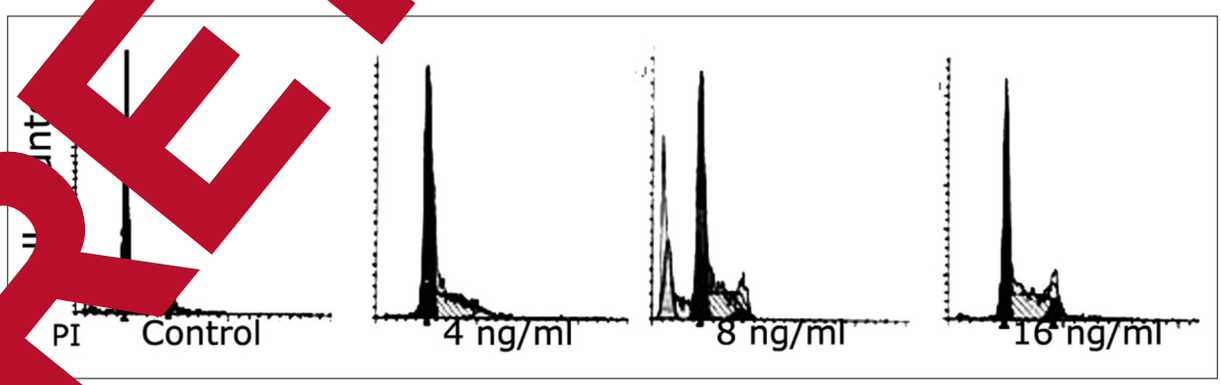

Figure 4. As_2O_3 blocked BGC-823 cell cycle.

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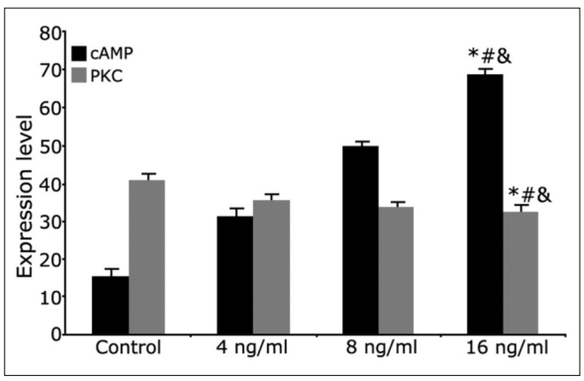

Figure 5. cAMP and PKC levels in GBC-823 cells. $^{*}p < 0.05$, compared with normal control. $^{\#}p < 0.05$, compared with 4 ng/ml group. $^{\&}p < 0.05$, compared with 8 ng/ml group.

indicated that As_2O_3 blocked BGC-823 cells in G0/G1 phase, thus to restrain cells entering S phase and suppress cell proliferation. Previous research proved that As_2O_3 could induce gastric cancer cell apoptosis and restrain cell cycle, resulting in gastric cancer cell necrosis and apoptosis and thus inhibiting cancer progre[14] cAMP, mainly activating protein kina found to affect the endogenous endonu se activity to achieve the purpose of apoptos duction[15,16]. PKC is a kind of protein kinase affects signaling pathway and is involved in va ious physiological and path rocesses, such as cell metabolism erent on, and proliferation[17,18]. Previou searche detected PKC and cAMP leve he c that PKC downregul ion P elevation obviously suppres cell pr ion and enhanced cell apo s. Our wor nstrated that As_2O_3 up cAMP level reduced PKC level i GC-8 ls. It was proved that cAMP an ogue or cA ducer significantly elevated MP concentrati he cells, which thus i ed cell apoptosis. Th induction effect of cAMP on cells may be mediated by both PKA and cAMP dependent protein which in turn activated correspondi spons element to exert its biological fun . It seems that as a differentiation inducer, duction of cAMP causes canceration. From th pective of treatment, rebuilding th vel of in the cells might be an effe strategy to the progression of th mor. r findi showed that As_2O_3 nced cAMP level and declined PKC in C-823 ls, which was prop l to of th echanisms of prom cell ap

Finally, w pted Weste to test the apoptosis teins expres n in BGC-823 cells, cludi detection of Bcl-2, Bax, and Survivin. As_2O_3 ent decreased Bcl-2 and n levels, and nced Bax expression. Bcl-2 has been confirmed to be negatively related with ap tosis, while shows no effect mitosis. As an portant apoptosis suppressor, is proved educe Bax expression level, th uppress ptosis through a series of signalin Survivin is the hotspot of antitumor treatment in clinic by involving in the regulation of cell apoptosis and regulating mitosis. ly suggested that As_2O_3 induced cell op through downregulating Bcl-2 and Survivin expression while enhancing Bax level in BGC-823 cells.

Conclusions

As_2O_3 induced gastric cancer cell apoptosis by blocking BGC-823 cells in G0/G1 phase, elevating cAMP level, reducing PKC level, downregulating Bcl-2 and Survivin expression, and enhancing Bax level. It may be treated as new leads for the discovery of As_2O_3 therapy on gastric cancer. Further in-depth investigation needed to clarify the specific mechanism.

Table ax, and vin protein expression analysis in BGC-823 cells treated by As_2O_3.

Group	Bcl-2	Bax	Survivin
iment			
ng/ml	0.53 ± 0.13*	0.16 ± 0.09*	0.53 ± 0.21*
16 ng/ml	0.27 ± 0.08*#	0.39 ± 0.11*#	0.29 ± 0.12*#
ntrol	0.14 ± 0.01*#&	0.53 ± 0.37*#&	0.18 ± 0.07 *#&
	0.89 ± 0.41	0.81 ± 0.57	0.72 ± 0.47

*p 05, compared with normal control. $^{\#}p < 0.05$, compared with 4 ng/ml group. $^{\&}p < 0.05$, compared with 8 ng/ml group.

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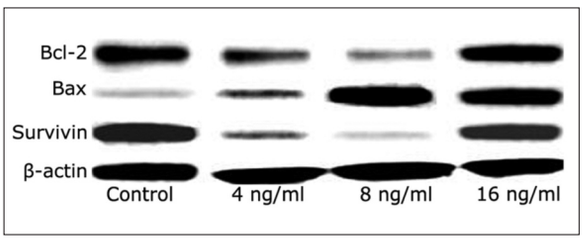

Figure 6. As_2O_3 affected Bcl-2, Bax, and Survivin protein expressions in BGC-823 cells.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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