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• ESOPHAGEAL CANCER •

The sensitivity of digestive tract tumor cells to As2O3 is associated with the inherent cellular level of reactive oxygen species

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Abstract

AIM: **To explore the correlation of the inherent cellular ROS level with the susceptibility of the digestive tract tumor cells to apoptosis inducted by As_2O_3.**

METHODS: **Two gastric carcinoma cell lines, SGC7901 and MKN45, and two esophageal carcinoma cell lines, EC/CUHK1(alternatively named EC1.71) and EC1867 with low concentration(2μmol·L^{-1})of As_2O_3 were cultured respectly, which confirmed the difference in apoptosis susceptibility between SGC7901 and MKN45, and between EC/CUHK1 and EC1867. The cells were incubated with dihydrogenrhodamine123 (DHR123), used as a ROS capture in absence of As_2O_3. The fluorescent intensity of rhodamine123, which was the product of cellular oxidation of DHR123, was detected by flow cytometry, and ROS was measured.**

RESULTS: **Apoptosis induced by a low concentration of As_2O_3 was more readily to occur in SGC7901 (22.4%±2.4%) and EC/CUHK1(27.0%±2.9%) than in MKN45(2.1%±0.5%) and EC1867(0.8%±0.5%). In other words, SGC7901 was more sensitive than MKN45 to As_2O_3, meanwhile EC/CUHK1 was more sensitive than EC1867 to As_2O_3. The level of inherent cellular ROS in SGC7901(650±37) was higher than that in MKN45(507±22)(P<0.01), and the level of inherent cellular ROS in EC/CUHK1(462±17) was higher than that in EC1867(187±12) (P<0.01).**

CONCLUSIONS: **The cellular sensitivity to apoptosis induced by As_2O_3 is associated with the difference in cellular ROS level. The inherent ROS level might determinate the apoptotic sensitivity of tumor cells to As_2O_3.**

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INTRODUCTION

Arsenic trioxide (As_2O_3) has proved to be effective in the treatment of acute promyelocytic leukemia (APL)[1-7]. While many researchers aimed at the effectiveness of As_2O_3-induced apoptosis on the other leukemic cells and some solid tumor cells, a lot of evidence showed that some types of tumor cells were sensitive while others were insensitive to apoptosis-inducing effect of As_2O_3[2,8-20]. Unraveling the causes of such sensitivity difference in the tumor cells will benefit not only the clinical selection of patients,to which As_2O_3 can be given, but also understanding the mechanisms underlying the apoptosis induced by As_2O_3.

Previously we investigated the sensitivity of a series of digestive tumor cell lines to As_2O_3. We identified that there were difference of sensitivity to apoptosis inductied by low concentration (2μmol/L) of As_2O_3 between the gastric carcinoma cell line SGC7901 and MKN45, and between the esophageal carcinoma cell line EC/CUHK1(alternatively named EC1.71) and EC1867; SGC7901 was more sensitive than MKN45, and EC/CUHK1 was more sensitive than EC1867 to As_2O_3[15-16]. We found that As_2O_3 induced cell apoptosis via directly influencing mitochondrion, consequently causing decrease of transmembrane potential and increase of reactive oxygen species (ROS) level[17]. Recently it was evidenced that ROS participate the apoptosis induction of acute promylocytic leukemia[18,19,21,22]. But whether the difference of sensitivity of digestive tumor cells to apoptosis-inducing effect of As_2O_3 is associated with the inherent cellular ROS level is not clearly understood. In this study, we demonstrated the difference between SGC7901 versus MKN45, and EC/CUHK1 versus EC1867, thereby explored the ralation between the sensitivity of cell to apoptosis induction of As_2O_3 and the inherent cellular ROS level.

MATERIALS AND METHODS

Cell Lines and Culture Conditions

Gastric carcinoma cell line SGC7901 *vs* MKN45, and esophageal carcinoma cell line EC/CUHK1 *vs* EC1867(kindly provided by professor Shen, Shantou University) were cultured in DMEM medium supplemented with 100kU·L^{-1} pennicillin,100mg·L^{-1} streptomycin, and 100mL·L^{-1} fetal bovine serum(Gibco) in a fully humidified atomosphere with 50mL·L^{-1} CO_2 at 37℃. Cells were split when reached to 80% confluency.

Inducing Cell Apoptosis by As_2O_3

About 5×10^5 tumor cells in logarithmic stage were treated with 2μmol·L^{-1} concentration of As_2O_3 (Sigma) for 72h and analyzed by flow cytometry and electron microscopy for apoptosis[15-17]. As_2O_3 powder was dissolved in small amounts of 1.0 mol·L^{-1} NaOH, then diluted to 10.0 mmol·L^{-1} with phosphate-buffered saline(PBS) as stock solutions.

Detection Inherent ROS Level

The cells was incubated with 1μmol·L^{-1}dihydrorhodamine123 (DHR123,Sigma), as a ROS capture[23-25], for 1 or 24h. Blank and positive controls were set, in which DHR123 was either omitted or plus 50μmol·L^{-1} of hydrogen peroxide (H_2O_2). DHR123 could be oxidized intracellularly to form the fluorescent compound rhodamine123 (Rh123) by ROS, and be pumped into mitochondria and remained there. After incubated with DHR123, cells were trypsinized and harvested before an immediate detection of

fluorescence intensity of Rh123 by flow cytometry FACscan (Becton Dickinson), and the cellular ROS level was thus measured.

RUSULTS

Cell Apoptosis Induced by As_2O_3

A significant apoptosis was observed in EC/CUHK1 and SGC7901 cells with 2μmol/L of As_2O_3 for 3 days while no remarkable apoptosis could be seen in EC1867 and MKN45 cells with the equivalent As_2O_3. The characteristic morphological changes were displayed in the apoptotic cells, including the shrinkage of the nuclear membrane, condensation and margination of the chromatin, and nuclear breakage (Figure1). DNA flow cytometry showed that the some cells with fractional DNA, as typical display of apoptosis, appeared, (27.0±2.9)% and (22.4±2.4)% ($\bar{x}$±s,n=5)respectively in EC/CUHK1 and SGC7901 cells, but hardly visible in EC1867 (0.8±0.5)% and MKN45 (2.1±0.5)%. (Figure 2).

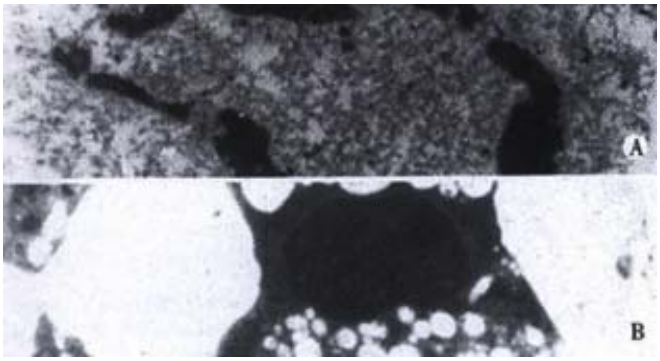

Figure 1 Apoptotic cells in EC/CUHK1 and SGC7901 with the condensation and margination of chromatin, and nuclear breakage EM×6000

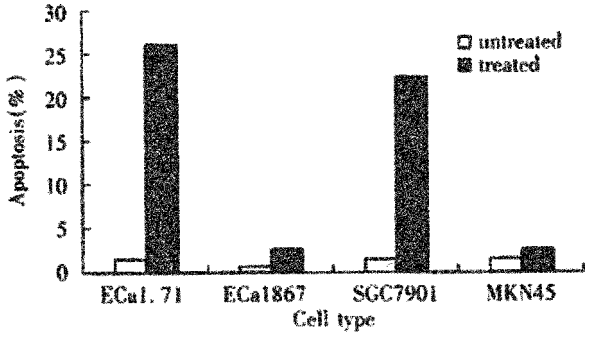

Figure 2 Flow cytometry with PI staining: apoptosis proportions in EC/CUHK1, EC1867, SGC7901 and MKN45

Inherent Cellular ROS Level

After incubation with DHR123 for 1 or 24h in absence of As_2O_3, the values ($\bar{x}$±s,n=3) of fluorescent intensity for Rh123 were 29±4. 1 and 650±37 in SGC7901 cells; 21±1.4 and 507±22 in MKN45 cells; 50±3.9 and 462±17 in EC/CUHK1; 46±6.4 and 187±12 in EC1867 cells. The fluorescent intensity in blank control was less than 3. The values for the positive controls (DHR123 plus hydrogen peroxide incubation for 1h) were 80±4.9 in SGC7901; 27±3.0 in MKN45; 72±5.8 in EC/CUHK1; and 19±2.1 in EC1867. Figure 3 displayed the fluorescence histograms for four types of cells after incubation with DHR123 for 24h. The data showed that,in absence of As_2O_3, the cellular ROS level was higher in SGC7901 than in MKN45, and higher in EC/CUHK1 than in EC1867. Such differences were augmented in 24h incubation as shown above, where the value in SGC7901 was as 1.3 times as in MKN45, and in EC/CUHK1 was 2.5 times as in EC1867.

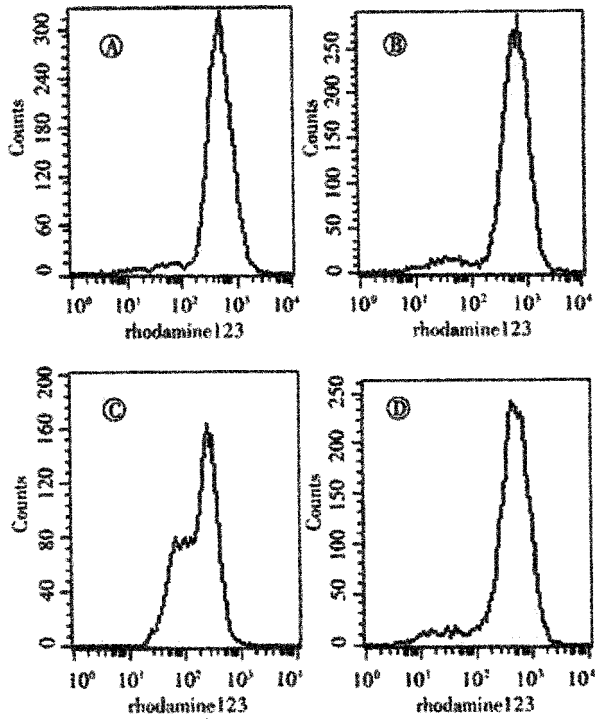

Figure 3 Flow cytometry displaying the inherent ROS level of cells A:MKN45; B:SGC7901; C:EC1867; D:EC/CUHK1

DISCUSSION

ROS, including superoxide anion (O^-_2), hydrogen peroxide(H_2O_2), hydroxyl free radical (OH) and singlet oxygen ($^1O_2$), continuously generated from mitochondrial respiratory chain, have powerfully oxidative potential. ROS is capable of attacking lipids, nuclear acids and proteins, resulting in certain degree of oxidative damages[26-35]. It has been thought recently to involve in apoptosis triggering and signaling[36-43]. Cell possesses an efficient antioxidant defense system, mainly composed of the enzymes such as superoxide dismutase, glutathione peroxidase, and catalase, which can scavenge the ROS excessive to cellular metabolism, and make ROS level relatively stable under physiological conditions[26-35]. Though it has been noticed that ROS were involved in As_2O_3-induced apoptosis[18,19,21,22], evaluation of ROS level differences directly by a flow cytometric detection of ROS, to our knowledge, has not been frequently reported. Instead, H_2O_2, a kind of ROS, was adopted to represent the total ROS level, usually judged from a decrease in activity of glutathione peroxidase or catalase, or a decrease in ratio of reductive/oxidative glutathione[18,19]. The total ROS level in the resting cells, however, was directly measured in the present study, by flow cytometric detection of Rh123. The comparative investigation on the inherent ROS levels in the cells showed that there were different apoptosis susceptibility to As_2O_3. In this study, inherent ROS level signified the basal cellular level of ROS in absence of any drug or exogenous ROS.

Detecting ROS level by flow cytometry has been a novel approach with characteristic of rapidness, convenience and reproducibility. DHR123, one of common ROS captures, is membrane permeable. It is oxidized by ROS intracellularly to become fluorescent Rh123, and is pumped into mitochondria and remain there, then is detectable by flow cytometry after a period of accumulation[23-25]. 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) is another agent used to capture ROS. It is

cleaved by nonspecific esterases to form DCFH, which was further oxidized to form the fluorescent compound DCF and kept inside cells[19,44,45]. It proved important, as we realized in this study, to prolong the incubation time with the ROS capture in order to visualize the nuance in ROS, since the absolute quantity of ROS is scarce. We selected two time intervals to visualize the accumulation of Rh123 fluorescence, finding that difference began to display at 1 h and became much pronounced by 24 h. These parameters definitely represented the difference of ROS level inherently existed in the respective types of cells. A similar result was obtained by using DCFH-DA in our study. Recently it was evidenced that NB4 leukemia cell line, which is sensitive to low concentration of As_2O_3 (1-2μmol/L), had higher H_2O_2 level than the U937 leukemia cell line which is insensitive to As_2O_3, and exposure of cells to low concentration of As_2O_3 elevated the level of H2O2 in NB4 but not in U937[19]. Though these studies indicated that a higher H2O2 level in NB4 might link to its higher sensitivity to As_2O_3 -induced apoptosis[19], whether there existed a difference in total ROS level between cell lines which possessed different susceptibility to As_2O_3 -induced apoptosis, prior to As_2O_3 treatment, has not been documented.

Based on our previous work, we selected two pairs of digistive tract cell lines EC/CUHK1 versus EC1867, SGC7901 versus MKN45 in which one type of cell was susceptible and the other type was unsusceptible to As_2O_3 -induced apoptsis in this study, and measured the inherent levels of total ROS in these cells. The data on both pairs showed that the inherent ROS level was higher in sensitive cells. These results indicated that difference in apoptosis susceptibility of tumor cells to low concentration of As_2O_3, was associated with the difference in the inherent cellular level of ROS, and what's more, the inherent ROS level might be pivotal in determination of the cellular susceptibility to As_2O_3 -induced apoptosis. The difference of inherent ROS level between cells probably resulted from the differential expression of enzymes involved in ROS generation and elimination[46-51]. An interference to the expression of relevant enzymes or simply ROS is likely an approach by which an improved effect and expanded usage of arsenic trioxide can be achieved clinically.

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