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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Activation of Signal Pathways of Apoptosis under Conditions of Prolonged ER-Stress Caused by Exposure of Mouse Testicular Teratoma Cells to Selenium-Containing Compounds

M. V. Goltyaev[a], E. G. Varlamova[a,*], S. V. Novoselov[a], and Corresponding Member of the RAS E. E. Fesenko[a]

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Abstract—*Aim* to study the molecular mechanisms of apoptotic death of mouse testicular teratocarcinoma cells (line F-9) under exposure to the widely used selenium-containing compounds with antitumor activity, sodium selenite and methylseleninic acid. *Methods* fluorescence microscopy, MTT assay, Western blotting. *Results* It was shown that sodium selenite at a concentration of 10 μM and methylseleninic acid at concentrations of 1 and 10 μM cause apoptosis-dependent death of F-9 cells, excluding necrotic death. Western blotting showed an increase in the expression of XBP1s when treating F-9 cells with 1 μM methylseleninic acid. *Conclusions* 10 μM methylseleninic acid leads to cell apoptosis, most likely by activation of the IRE1 signaling pathway under prolonged stress of the endoplasmic reticulum.

Keywords: ER-stress, sodium selenite, methylseleninic acid, carcinogenesis

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Sodium selenite (SS) and methylseleninic acid (MSA) are widely used potential anticancer selenium-containing compounds, which was repeatedly confirmed in a number of studies [1–11]. It is known that the cell death under the influence of SS and MSA is associated with ER-stress [1, 2, 11, 12], which is related to the signaling pathways united by a common trigger mechanism and represented by the triad of transmembrane proteins: PERK, IRE1, and ATF6. The purpose of this study was to investigate the changes in the expression of the key members ATF4 and XBP1s of two signaling pathways, PERK and IRE1, respectively, by Western blotting analysis after the treatment of F-9 cancer cells (mouse testicular teratocarcinoma) with SS and MSA at concentrations of 1 and 10 μM.

We have previously found that the treatment of F-9 cells with 1 and 10 μM SS decreased their viability [12]. However, no similar experiments with MSA have been performed. To establish the pathway of death (apoptosis/necrosis) of the studied cells after their 24-h exposure to SS and MSA at concentrations of 1 and 10 μM, we used the Apoptosis/Necrosis Detection Kit (Abcam, United States) in tandem with fluorescence microscopy. This fluorescence analysis makes it possible to detect live cells (blue, stained with CytoCalcein Violet 450), apoptotic cells (green, stained with Apopxin Green), and necrotic cells (red, stained with 7-AAD). Before treatment with the selenium-containing compounds, F-9 cells were seeded in a 96-well plate in DMEM medium containing 10% serum and grown in a CO_2 incubator to a density of 5×10^5 cells/well for 24 h, after which SS and MSA were added to the cells at appropriate concentrations. After incubation for 24 h, the cells were resuspended in the assay buffer according to the manufacturer's protocol. The apoptotic cells were detected using an Apopxin Green Indicator and visualized using the FITC channel (Ex/Em = 490/525 nm). The healthy cells were detected using CytoCalcein 450 (Ex/Em = 405/450 nm), and the necrotic cells were detected using 7-aminoactinomycin D (Ex/Em = 550/650 nm).

Figure 1 shows the results of qualitative analysis, which provide information about the presence/absence of apoptosis or necrosis in the cancer cells studied. They show that, after a 24-h treatment with 10 μM SS and 10 μM MSA, apoptosis was observed in all visualized cells (100%). After the treatment of cells with 1 μM SS, the majority of cells did not lose their adhesive properties and retained viability, although their morphology changed (cells acquired spherical shape). Interestingly, when cells were treated with 1 μM MSA, approximately 50% of the cells underwent apoptosis, whereas the remaining 50% remained viable.

To establish the signaling pathways that are activated as a result of the treatment of cells with SS and

[a] *Institute of Cell Biophysics of the Russian Academy Sciences, Pushchino, Moscow Region, Russia*
**e-mail: 1928lv@mail.ru*

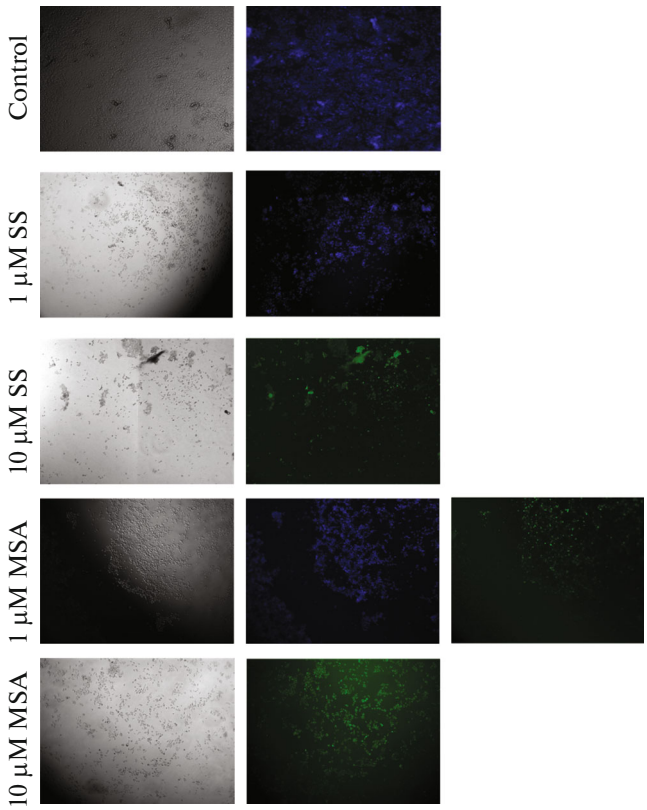

Fig. 1. Fluorescent analysis of the viability of F-9 cancer cells after their 24-h exposure to SS and MSA at concentrations of 1 and 10 μM. The live cells were stained with CytoCalcein Violet 450 (blue), and the apoptotic cells were stained with Apopxin Green Indicator (green).

MSA in each case, we tested how the expression of their key components changed by Western blotting analysis. For this purpose, cells were exposed to 1 and 10 μM SS and MSA for 24 h, after which they were lysed with the buffer PBS 1 (pH 7.4) containing 1 mM PMSF. The total protein content in each sample was measured colorimetrically by the method of Bradford. Proteins were separated by SDS-PAGE in 12% polyacrylamide gel. The content of proteins in each sample (cell lysate) was 50 μg. We used the commercial primary antibodies against the transcription factor ATF4, XBP1 (X-box binding protein 1), and the reference protein GAPDH at a dilution of 1 : 200 and the secondary antibodies conjugated to horseradish peroxidase (Abcam, United States) at a dilution of 1 : 2000 (Fig. 2).

Proteins were quantitated using the Fusion Solo chemiluminescence documentation system (Viber, France). This experiment was repeated three times (Fig. 3). It was found that, when cells were treated with 1 μM MSA, the expression of the spliced form XBP1s significantly increased, which may indicate the activation of the IRE1 signaling pathway. The XBP1 transcription factor is involved in the expression regulation of the genes that are required for the immune system functioning and in the regulation of response to ER-stress. Activated IRE1 (inositol-requiring enzyme 1) catalyses the elimination of the noncanonical intron (26 nucleotides) from the ubiquitously expressed XBP1u mRNA (unspliced XBP1, 29.5 kDa), which causes a frame shift in the coding sequence XBP1 and translational of the XBP1s isoforms (spliced XBP1, 55 kDa).

In all other samples of cells treated with both SS and MSA, the expression level of XBP1u and XBP1s was approximately the same. In the case of activation of the PERK signaling pathway, detection of an increase in the expression of the ATF4 transcription factor induced by phosphorylation of eIF2α, the key component of protein translation, could be expected. However, after the treatment of cells with both SS and MSA at the studied concentrations, the expression level of ATF4 did not differ from the control (intact F-9 cancer cells). Possibly, the apoptosis of cancer cells, in which no increase in the expression of either ATF4 or XBP1s was observed, was caused by the activation of

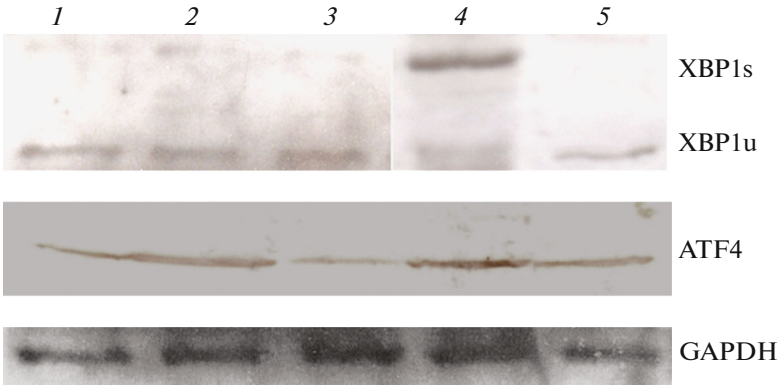

Fig. 2. Western blot analysis of protein expression (XBP1s, ATF4 and GAPDH) in F-9 cancer cell lysates after 24-h exposure to SS and MSA at concentrations of 1 and 10 μM by immunoblotting: (*1*) protein content in intact cells and protein content after exposure of cells to (*2*) 1 μM SS, (*3*) 10 μM SS, (*4*) 1 μM MSA, and (*5*) 10 μM MSA.

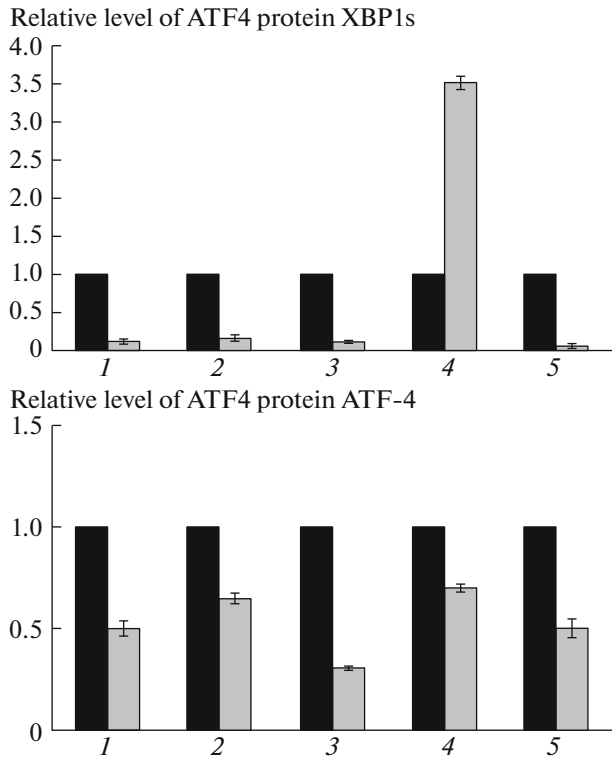

Fig. 3. Quantification of XBP1s and ATF4 proteins with respect to GAPDH in F-9 tumor cell lysates after their 24-h exposure to SS and MSA: (*1*) protein content in intact cells and protein content after exposure of cells to (*2*) 1 μM SS, (*3*) 10 μM SS, (*4*) 1 μM MSA, and (*5*) 10 μM MSA.

the third signaling pathway ATF6. This requires a more detailed analysis and confirmation in further studies.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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