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THE IMPACT OF GRAPHENE OXIDE NANOPARTICLES ON THE EXPRESSION OF ENDOPLASMIC RETICULUM STRESS-DEPENDENT GENES IS MORE PRONOUNCED IN NORMAL HUMAN ASTROCYTES THAN GLIOBLASTOMA CELLS

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Graphene and its derivatives, due to a wide range of unique properties that they possess, can be used as initial material for the synthesis of nanocomplexes for advanced therapeutic strategies, targeted delivery of chemotherapeutic agents or cellular-sensing probes [1, 2]. Graphene oxide (GO), as water-soluble derivative of graphene, can pass through the blood-brain barrier and induce cytotoxicity in the central nervous system, which is important for glioblastoma therapy [3, 4]. However, there is a risk that the use of graphene oxide nanoparticles in cancer therapy will also pose a risk to healthy cells.

Aim. To investigate the impact of graphene oxide nanoparticles on the expression of a subset of ER stress-dependent genes associated with cell proliferation and carcinogenesis in normal human astrocytes in comparison with glioblastoma cells depending on the knockdown of ERN1.

Materials and Methods. The immortalized normal human astrocytes (NHA/TS cell line) and the sublines of U87MG with native and knockdown of ERN1 were growing as described previously [5]. The culture plates with normal human astrocytes and glioblastoma cells were exposed to two doses of GO nanoparticles (1 and 4 ng/ml of medium) for 24 h. GO (2 mg/ml, dispersion in water) was received from Sigma-Aldrich Chemie GmbH, Germany. Total RNA was extracted from normal human astrocytes and glioblastoma cells using the TRIzol reagent according to the manufacturer's protocol as described previously [6]. The expression level of genes related to cell proliferation was studied by real-time qPCR in normal human astrocytes line NHA/TS (Cambrex Bio Science, Walkersville, MD, USA) using SYBRGreen Mix and specific for each mRNA forward and reverse primers as described previously [7]. The values of mRNA expressions were normalized to the level of ACTB mRNA and represented as percent of control (100%).

Results and Discussion. As shown in the Figure, GO nanoparticles strongly upregulated the expression level of ATF3, ATF4 and TOB1 mRNA in both normal human astrocytes and glioblastoma cells, but normal cells were more sensitive to the genotoxic action of GO nanoparticles than glioblastoma cells.

Thus, the results of this investigation demonstrated that GO nanoparticles at relatively low concentrations dysregulate the expression of genes encoding various important regulatory factors related to ER stress, cell proliferation, and carcinogenesis in normal human astrocytes much stronger than glioblastoma cells and that changes in studied gene expressions possibly reflect the genotoxic and neurotoxic effects of these unique carbon nanoparticles. Moreover, most of the observed changes in the expression of studied genes in both normal human astrocytes and glioblastoma cells may be mediated by ER stress signaling pathways introduced with GO nanoparticles like many other nanoparticles [6, 8–10].

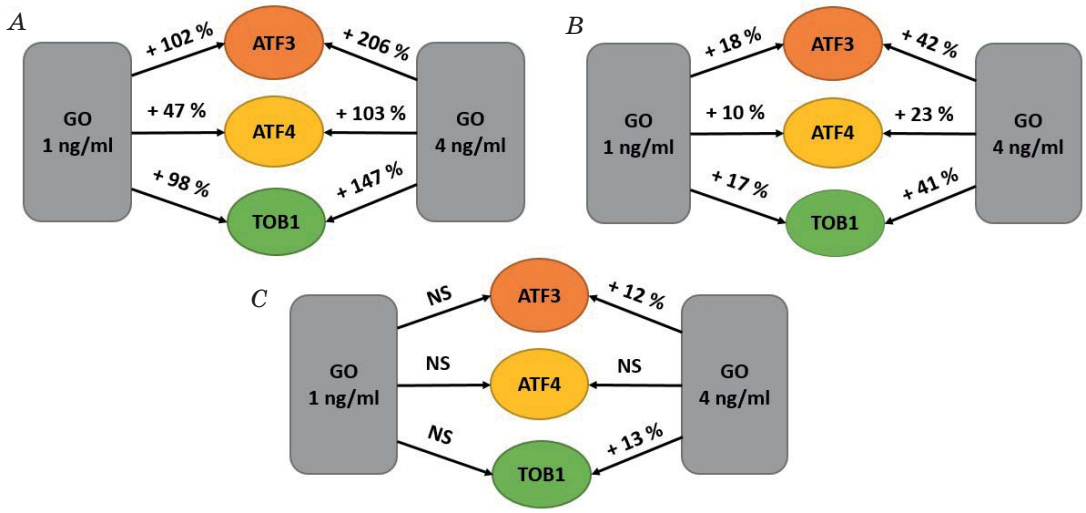

Fig. **Schematic representation of the dose-dependent impact of graphene oxide on the expression level mRNA in normal human astrocytes NHA/TS (*A*), U87MG glioblastoma cells stable transfected by empty vector (U87-Vector) (*B*) and with a dominant-negative ERN1 construction (U87-dnERN1) (*C*)**

Conclusions. GO nanoparticles, which are considered as promising theranostic agents, especially in cancer therapy, exert a more pronounced dose-dependent impact on the expression of genes responsible for ER stress, cell proliferation, and cancerogenesis in normal human astrocytes than glioblastoma cells. In this case the polyresistance of cancer cells may be associated with the induction of long-term ER stress. At the same time, the activation of ER stress in normal cells may indicate the occurrence of genotoxic effects due to the action of graphene oxide nanoparticles.

Key words: graphene oxide, mRNA expression, genotoxicity, normal human astrocytes, glioblastoma cells, ERN1 knockdown.

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