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The expression of DNAJB9 in normal human astrocytes is more sensitive to nanographene oxide than in glioblastoma cells

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Objective. Nanographene oxide (nGO) nanoparticles (NPs) have unique properties and are widely used in various fields, including biomedicine. These NPs, however, also exhibit toxic effects and therefore, the understanding of the molecular mechanism of nGO toxicity is very important mainly for the nanomedicine, especially the cancer therapy. This study aimed to examine the impact of nGO NPs on the expression of genes associated with endoplasmic reticulum (ER) stress, proliferation, and cancerogenesis in both normal human astrocytes and U87MG glioblastoma cells.

Methods. Normal human astrocytes line NHA/TS and U87MG glioblastoma cells stable transfected by empty vector or dnERN1 (dominant-negative construct of ERN1) were exposed to low doses of nGO (1 and 4 ng/ml) for 24 h. RNA was extracted from the cells and used for cDNA synthesis. The expression levels of DNAJB9, EDEM1, DDIT3, ATF3, ATF4, TOB1, and IDH2 mRNAs were measured by quantitative polymerase chain reaction and normalized to ACTB mRNA.

Results. We showed that treatment of normal astrocytes and glioblastoma cells by relatively small doses of nGO (1 and 4 ng/ml for 24 h) affected the expression level of DNAJB9, EDEM1, DDIT3, ATF3, ATF4, TOB1, and IDH2 mRNAs, but the sensitivity of all studied mRNA expressions to these NPs was significantly higher in normal astrocytes than in glioblastoma cells. The impact of nGO on these gene expressions is mediated by ER stress because ERN1 knockdown suppresses the effect of these nanoparticles in glioblastoma cells.

Conclusion. The data obtained demonstrate that the low doses of nGO disturbed the functional integrity of the genome preferentially through ER stress signaling and exhibit a more pronounced genotoxic effect in the normal astrocytes than the glioblastoma cells.

Keywords: nanographene oxide, DNAJB9, DDIT3, EDEM1, IDH2, mRNA expression, genotoxicity, ERN1 knockdown, normal human astrocytes, glioblastoma cells

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 nano complexes for advanced therapeutic strategies, i.e. targeted delivery of chemotherapeutic agents or cellular-sensing probes (Tonelli et al. 2015; Li et al. 2021a; Cellot et al. 2022; Itoo et al. 2022). The water-soluble derivative of graphene, graphene oxide (GO) nanoparticles (NPs), has been widely applied in numerous fields, especially in biomedical applications including cancer treatment (Avitabile et al. 2018; Sharma and Mondal 2020; Dash et al. 2021). At the same time, the hazardous impact of these NPs on human health and the environment has also been shown (Fadeel et

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al. 2018; Jia et al. 2019; Chen et al. 2020; Malhotra et al. 2020; Cruces et al. 2021; Rhazouani et al. 2021). Nanographene oxide (nGO) can pass through the blood-brain barrier and induce cytotoxicity in the central nervous system, which is important in glioblastoma therapy (Mendonca et al. 2015; Tonelli et al. 2015; Dong et al. 2016; Cui et al. 2021; Dash et al. 2021; Liu et al. 2022). Recently, we have demonstrated that nGO in relatively small concentrations disturbed the genome function affecting the expression of key regulatory genes in a gene-specific manner showing a genotoxic effect on normal human astrocytes (Rudnytska et al. 2022).

At the same time, precise molecular mechanisms of GO action and toxicity are still largely unknown, especially in normal astrocytes, the most abundant cells in the brain. Like other NPs, nGO NPs may affect many intracellular pathways including endoplasmic reticulum (ER) stress signaling pathways (Chen et al. 2014; Barberet et al. 2017; Cao et al. 2017; Simon et al. 2017; Minchenko et al. 2023). Moreover, suppression of ER stress with 4-phenylbutyric acid inhibited most effects of titanium dioxide NPs (Hu et al. 2018). We have shown that the knockdown of ERN1 (ER to nucleus signaling 1) signaling pathway of ER stress in U87MG glioblastoma cells led mainly to the elimination of the impact of relatively small doses of single-walled carbon nanotubes on *DNAJB9* and many other gene expressions (Minchenko et al. 2023). Thus, ER stress plays an important role in molecular mechanisms of different NP genotoxicity.

In this study, we investigated the impact of relatively low concentrations of nGO on the expression of *DNAJB9* (DnaJ heat shock protein family (Hsp40), member B9), *DDIT3* (DNA-damage-inducible transcript 3) also known as CHOP (CCAAT/enhancer-binding protein homologous protein), *EDEM1* (ER degradation enhancing alpha-mannosidase like protein 1), *ATF4* (activating transcription factor 3), *ATF3*, *TOB1* (transducer of ERBB2, 1), and *IDH2* (isocitrate dehydrogenase 2) genes in normal human astrocytes (NHA/TS cell line) and glioblastoma cells (U87MG cell line). These genes encode polyfunctional proteins, which participate in ER stress signaling and cancerogenesis and also play an important role in the regulation of multiple metabolic processes. Proteins, encoded by the *DNAJB9* gene represent cell stress chaperone, have pro-proliferative properties and are increased in different cancers (Lee et al. 2015; Amin-Wetzel et al. 2017; Kim et al. 2021). Protein DNAJB9 is the ER luminal co-chaperone ERdj4, which is a selective ERN1 repressor. It promotes a complex between the ER chaperone BiP (binding immunoglobulin protein), which represents heat shock protein family A (Hsp70) member 5 (HSPA5), and the luminal stress-sensing domain of ERN1 (Amin-Wetzel et al. 2017). DNAJB9 is associated with ER stress and plays a role in protecting stressed cells from apoptosis through inhibition of p53, its pro-apoptotic function (Lee et al. 2015).

The EDEM1 protein represents an important quality control factor directly involved in the ER-associated degradation (ERAD) process and the alteration of its functions enhances ATF6 pro-survival signaling (Papaioannou et al. 2018; Chiritoiu et al. 2020). Transcription factor ATF3 is induced by ER stress and acts as a principal regulator of metabolic homeostasis (Ku and Cheng 2020). It plays very important role in controlling the metabolism and oncogenesis (Ku and Cheng 2020; Li et al. 2021b; Perrone et al. 2023). This transcription factor is also involved in the complex process of the cellular adaptive-response network including unfolded protein response, which can act as both a transcriptional activator and a repressor (Ku and Cheng 2020). It has also been shown that ATF3 can enhance breast cancer metastasis (Wolford et al. 2013). The ATF4 is also a stress-induced transcription factor that regulates the gene expression in ER stress responses including DDIT3, participates in the control of glucose homeostasis, and is often upregulated in cancer cells (Rozpedek et al. 2016; Wortel et al. 2017; Tang et al. 2020; Tao et al. 2023). Although stress-induced activation of ATF4 predominantly promotes cell survival, it can also enhance apoptosis (Wortel et al. 2017). Transcription factor DDIT3 participates in the control of TNF-alpha expression and suppression of breast cancer cell growth (Li et al. 2015; Block et al. 2019; Tang et al. 2020; Akhter et al. 2023). Decreased expression of TOB1, a transducer of ERBB2, and increased phosphorylation of this protein in nuclei promotes gastric cancer through control of the activity of the AKT/mTOR signaling pathway (Guan et al. 2017; Wang et al. 2022). Multifunctional protein IDH2 plays a role in intermediary metabolism and energy production and is critical for cell proliferation. Abnormal expression of IDH2 and its mutations have been identified in numerous types of cancer including gliomas (Lv et al. 2012; Babakhanlou et al. 2023; Shigeta et al. 2023).

In this study, we aimed to investigate the impact of nGO on the expression of a subset of ER stress-dependent genes associated with the cell proliferation and cancerogenesis in normal astrocytes and glioblastoma cells.

Materials and Methods

Experimental material. Nanographene oxide (2 mg/ml, dispersed in water) was received from Sigma-Aldrich Chemie GmbH (Germany). Trizol reagent was obtained from Invitrogen (Carlsbad, CA, USA). Thermo Scientific Versco cDNA Synthesis Kit was received from Thermo Fisher Scientific (Waltham, MA, USA). Luna Universal qPCR Master Mix was received from New England BioLabs, Inc. (Ipswich, MA, USA) and SYBR Safe DNA Gel Stain was from Life Technologies (Carlsbad, CA, USA).

Cell culture and treatment. The immortalized normal human astrocytes (NHA/TS cell line) were received from Drs. K. Sasai and S. Tanaka (Japan) and grown at 37°C under a humidified atmosphere of 95% air and 5% CO_2 as reported previously (Sasai et al. 2007). The sublines of U87MG with native and knockdown of ERN1 and their growing were described previously (Auf et al. 2013). The culture plates with normal human astrocytes and glioblastoma cells were exposed to 2 doses of nGO (1 and 4 ng/ml of medium) for 24 h.

RNA extraction and cDNA synthesis. Total RNA was extracted from normal human astrocytes and glioblastoma cells using the TRIzol reagent according to the manufacturer's protocol as described previously (Minchenko et al. 2024). The RNA pellets were washed with 75% ethanol and dissolved in nuclease-free water. For additional purification, the RNA samples were re-precipitated with 95% ethanol containing sodium acetate and the resulting pellets were dissolved again in nuclease-free water. RNA concentration as well as its spectral characteristics was measured by NanoDrop One (Thermo Scientific). Reverse transcription of the obtained RNA was carried out using a Thermo Scientific Verso cDNA Synthesis Kit according to the manufacturer's protocol.

Real-time quantitative polymerase chain reaction. The expression level of *DNAJB9*, *DDIT3*, *EDEM1*, *ATF3*, *ATF4*, *TOB1*, *IDH2*, and *ACTB* genes was measured by real-time polymerase chain reaction (qPCR) using Luna Universal qPCR Master Mix and "QuantStudio 5 Real-Time PCR System" (Applied Biosystems). Quantitative polymerase chain reaction was performed in triplicates. The pair of primers specific for each studied gene was received from Sigma-Aldrich (St. Louis, MO, USA) (Table 1). The quality of amplification products was analyzed by melting curves and by electrophoresis using 3% agarose gel and visualized by SYBR* Safe DNA Gel Stain. An analysis of quantitative PCR was performed using a special computer program "Differential Expression Calculator". The values of different mRNA expressions were normalized to the level of beta-actin mRNA and represented as percent of control (100%).

Statistical analysis. All values are expressed as mean±SEM from triplicate measurements performed in four independent experiments. Statistical analysis was performed using the GraphPad Prism 8.0.1

Table 1
Characteristics of the primers used for quantitative real-time polymerase chain reaction

Gene symbol	Gene name	Primer's sequence	Nucleotide number in sequence	GeneBank accession number
DNAJB9	DnaJ heat shock protein family (Hsp40) member B9	F: 5'-gtcggagggtgcaggatatt R: 5'-tcagggtggtacttcatggc	217–236 407–388	NM_012328.3
DDIT3	DNA-damage-inducible transcript 3	F: 5'-cattgcctttctccttcggg R: 5'-ccagagaagcagggtcaaga	351–370 518–499	NM_001195053
EDEM1	ER degradation enhancing alpha-mannosidase like protein 1	F: 5'-tggaaacgatatggtgccct R: 5'-tctccatccggtcttctgtg	1495–1514 1741–1722	NM_014674.3
ATF4	Activating transcription factor 4	F: 5'-gtccctccaacaacagcaag R: 5'-actttctgggagatggccaa	1093–1112 1328–1309	NM_001675.4
ATF3	Activating transcription factor 3	F: 5'-ttgccatccagaacaagcac R: 5'-acactttccagcttctccga	314–333 545–526	NM_001674.4
TOB1	Transducer of ERBB2, 1	F: 5'-agcccgaacaagatcactca R: 5'-cacgtctcctgggaagctta	293–312 516–497	NM_005749.4
IDH2	Isocitrate dehydrogenase 2 (NADP+), mitochondrial	F: 5'-ccatgccgaagttcagatgg R: 5'-gtgaggccgcatagtcaaag	781–800 1013–994	NM_002168.4
ACTB	beta-actin	F: 5'-catccgcaaagacctgtacg R: 5'-cctgcttgctgatccacatc	948–967 1165–1146	NM_001101.5

program. A value of $p<0.05$ was considered statistically significant. All experimental qPCR data were analyzed for the normality of distribution using a graphical tool (normal probability plot) and a histogram as described previously (Rudnytska et al. 2021). A normal distribution of all analyzed data sets was shown. For analysis of some data sets the D'Agostino-Pearson test (p-value was close to 1.00) and the Shapiro-Wilk test ($W>0.9$; $p>0.9$) were used.

Results

The effect of nGO on the expression of genes encoding important regulatory factors associated with ER stress, cell proliferation, and cancerogenesis as well as some other cellular functions in normal human astrocytes and glioblastoma cells was studied. As shown in Figure 1, the exposure of normal human astrocytes to nGO (1 and 4 ng/ml of medium) led to a strong and dose-dependent upregulation of *DNAJB9* gene expression by 126% and 203%, correspondingly, as compared to the control. At the same time, the impact of nGO NPs on the expression of *DNAJB9* was significantly lower (+41% and +82%, respectively for 1 and 4 ng/ml of nGO), in glioblastoma cells with native ERN1 (Figure 1). Knockdown of ERN1 in glioblastoma cells reduced the effect of a lower dose of nGO (1 ng/ml) on *DNAJB9* gene expression up to control level; however, a lager dose of nGO NPs (4 ng/ml) reduced the expression level of *DNAJB9* gene even more (below the control by 14%) (Figure 1).

As shown in Figure 2, the expression of *EDEM1*, another ER stress-dependent gene, was less sensitive to genotoxic action of nGO as compared to *DNAJB9* gene expression. Thus, exposure of normal human astrocytes with nGO NPs (1 and 4 ng/ml of medium) led to an upregulation of *EDEM1* gene expression by 38% and 81%, respectively, as compared to the control cells. Much smaller changes in the expression of this gene were observed in glioblastoma cells with native ERN1 treated by nGO (+11% by 1 ng/ml and +31% by 4 ng/ml of these carbon NPs. At the same time, the effect of both doses of nGO on *EDEM1* gene expression was completely removed in glioblastoma cells with knockdown of ERN1 (Figure 2).

We also studied the effect of nGO on the expression of three ER stress-dependent transcription factors in both normal human astrocytes and glioblastoma cells with intact and knockdown ERN1. It was shown that the expression of the *DDIT3/CHOP* gene is very sensitive to the toxic action of these carbon

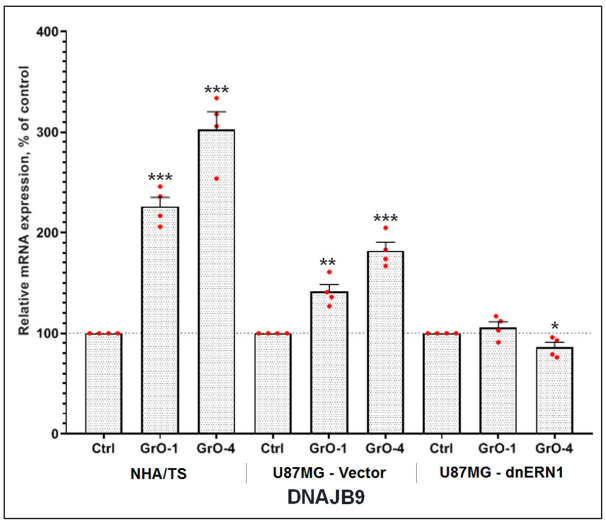

Figure 1. The impact of graphene oxide nanoparticles in doses 1 and 4 ng/ml (GrO-1 and GrO-4, respectively) for 24 h on the expression of DNAJB9 mRNA in normal human astrocytes (NHA/TS) and U87MG glioblastoma cells with native ERN1 (U87MG - Vector) and with knockdown ERN1 (U87MG - dnERN1). Values of this mRNA expression were normalized to ACTB mRNA expression. Ctrl – control cells. Data are presented as mean±SEM (n=4). **p<0.01; ***p<0.001 vs. corresponding control.

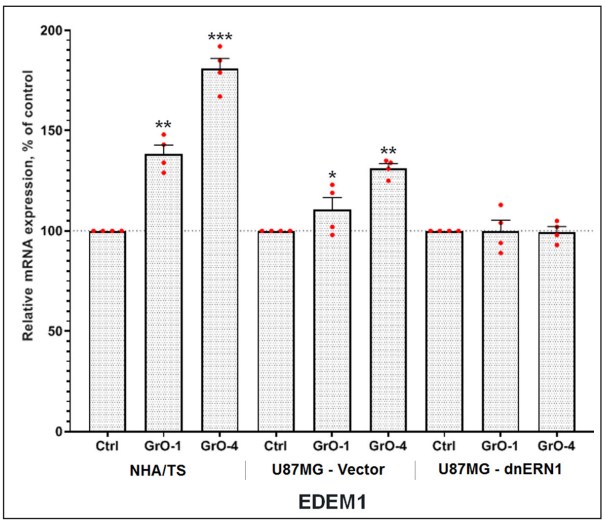

Figure 2. The impact of graphene oxide nanoparticles in doses 1 and 4 ng/ml (GrO-1 and GrO-4, respectively) for 24 h on the expression of EDEM1 mRNA in normal human astrocytes line NHA/TS (normal astrocytes) and U87MG glioblastoma cells with native ERN1 (glioblastoma cells) and knockdown ERN1 (ERN1 knockdown glioblastoma cells). Values of this mRNA expression were normalized to ACTB mRNA expression. Ctrl – control cells. Data are presented as mean±SEM (n=4). *p<0.05; **p<0.01; ***p<0.001 vs. corresponding control.

NPs (Figure 3). Its expression level was upregulated in normal human astrocytes by 139% and 211% by 1 and 4 ng of nGO per 1 ml of medium, respectively. Significantly smaller changes in *DDIT3* gene expression were detected in glioblastoma cells treated by nGO NPs: +62% by 1 ng/ml and +84% by 4 ng/ml (Figure 3). However, the ERN1 knockdown of glioblastoma cells removed the effect of smaller dose of nGO (1 ng/ml) on *DDIT3* gene expression and led to small, but statistically significant suppression of the expression of this gene (–13%) by larger dose (4 ng/ml) of nGO NPs (Figure 3).

As shown in Figure 4, nGO NPs affect also the expression of transcription factor ATF3, which is induced by ER stress, acts as a principal regulator of metabolic homeostasis and plays an important role in oncogenesis (Ku and Cheng 2020; Li et al. 2021b; Perrone et al. 2023; Wolford et al. 2013). Thus, nGO NPs strongly upregulated the expression of *ATF3* gene in both normal human astrocytes and glioblastoma cells, but normal cells were more sensitive to the genotoxic action of nGO than the glioblastoma cells (Figure 4). The expression level of *ATF3* gene was upregulated in normal human astrocytes by 100% and 198% by 1 and 4 ng/ml of nGO, respectively, and in glioblastoma cells by 27% (1 ng/ml) and 51% (4 ng/ml). But ERN1 knockdown removed the effect of both lower and higher doses of nGO NPs on ATF3 gene expression (Figure 4).

We also studied the impact of nGO on the expression of *ATF4* gene, which is also a stress-induced transcription factor that regulates the gene expression in ER stress responses including DDIT3 and is often upregulated in cancer cells (Wortel et al. 2017; Rozpedek et al. 2016; Tang et al. 2020; Tao et al. 2023). As shown in Figure 5, the genotoxic action of nGO on *ATF4* gene expression is significantly smaller compared to the *ATF3* gene. The exposure of normal human astrocytes to nGO led to upregulation of *ATF4* gene expression by 41% (1 ng/ml) and 98% (4 ng/ml). Much smaller changes in the expression of this gene were observed in treated by nGO glioblastoma cells with native ERN1 (+14% by 1 ng/ml and +36% by 4 ng/ml) of these carbon NPs (Figure 5). At the same time, the knockdown of ERN1 in glioblastoma cells completely removed the impact of nGO NPs on *ATF4* expression (Figure 5).

Other study demonstrates the effect of nGO on the expression of *TOB1* and *IDH2* genes, which have relation to the control of cell proliferation and cancerogenesis (Lv et al. 2012; Guan et al. 2017; Wang et al. 2022; Babakhanlou et al. 2023; Shigeta et al. 2023). As

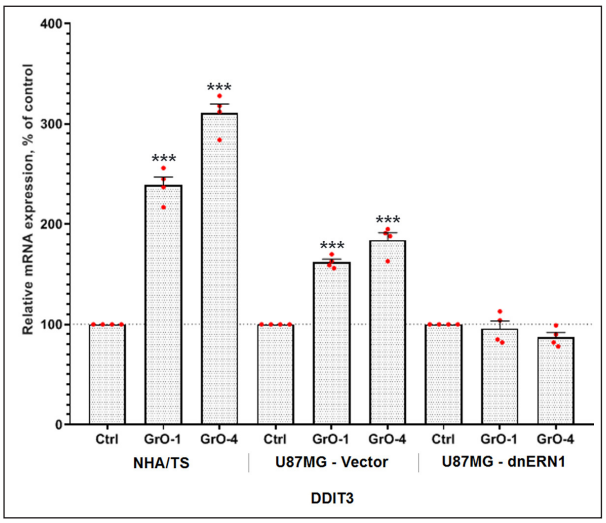

Figure 3. The impact of graphene oxide nanoparticles in doses 1 and 4 ng/ml (GrO-1 and GrO-4, respectively) for 24 h on the expression of DDIT3/CHOP mRNA in normal human astrocytes line NHA/TS (normal astrocytes) and U87MG glioblastoma cells with native ERN1 (glioblastoma cells) and knockdown ERN1 (ERN1 knockdown glioblastoma cells). Values of this mRNA expression were normalized to ACTB mRNA expression. Ctrl – control cells. Data are presented as mean±SEM (n=4). ***p<0.001 vs. corresponding control.

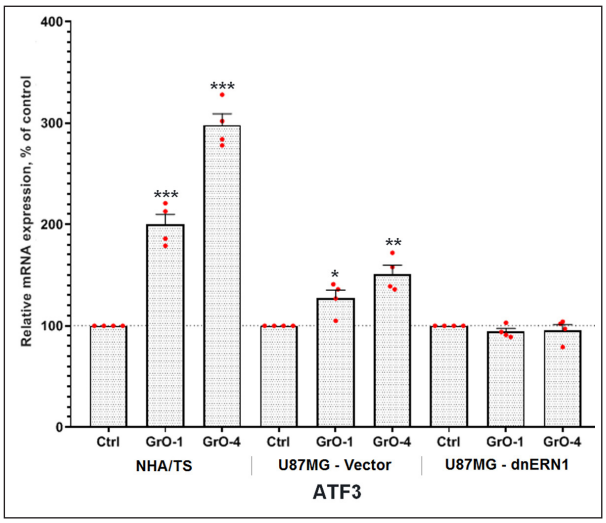

Figure 4. The impact of graphene oxide nanoparticles in doses 1 and 4 ng/ml (GrO-1 and GrO-4, correspondingly) for 24 h on the expression of ATF3 mRNA in normal human astrocytes line NHA/TS (normal astrocytes) and U87MG glioblastoma cells with native ERN1 (glioblastoma cells) and knockdown ERN1 (ERN1 knockdown glioblastoma cells). Values of this mRNA expression were normalized to ACTB mRNA expression. Ctrl – control cells. Data are presented as mean±SEM (n=4). *p<0.05; **p<0.01; ***p<0.001 vs. corresponding control.

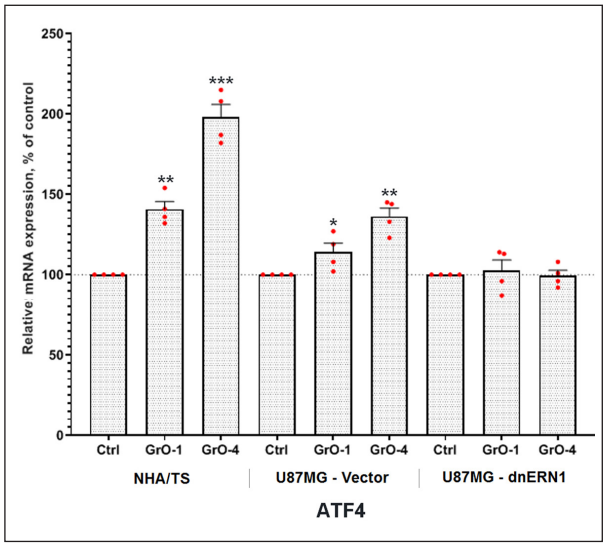

Figure 5. The impact of graphene oxide nanoparticles in doses 1 and 4 ng/ml (GrO-1 and GrO-4, respectively) for 24 h on the expression of ATF4 mRNA in normal human astrocytes line NHA/TS (normal astrocytes) and U87MG glioblastoma cells with native ERN1 (glioblastoma cells) and knockdown ERN1 (ERN1 knockdown glioblastoma cells). Values of this mRNA expression were normalized to ACTB mRNA expression. Ctrl – control cells. Data are presented as mean±SEM (n=4). *p<0.05; **p<0.01; ***p<0.001 vs. corresponding control.

shown in Figures 6 and 7, nGO strongly upregulated the expression of the *TOB1* gene and suppressed the *IDH2* gene in a dose-dependent manner. The exposure of normal human astrocytes to nGO led to strong upregulation of *TOB1* gene expression by 86% (1 ng/ml) and 175% (4 ng/ml). Much smaller changes in the expression of this gene were observed in glioblastoma cells with native ERN1 (+15% by 1 ng/ml and +41% by 4 ng/ml) treated by nGO (Figure 6). At the same time, the knockdown of ERN1 in glioblastoma cells completely removed the impact of these carbon NPs in smaller dose (1 ng/ml) on *TOB1* expression and significantly reduced the effect of larger dose (4 ng/ml) up to +12% (Figure 6). The opposite effect of nGO on gene expression was shown for IDH2 (Figure 7). The treatment of normal human astrocytes by nGO NPs led to the downregulation of *IDH2* gene expression by 41% (1 ng/ml) and 62% (4 ng/ml), but in glioblastoma cells with native ERN1, the changes in the expression of this gene were much smaller: –14% (1 ng/ml) and –30% (4 ng/ml). At the same time, the effect of nGO on *IDH2* expression was completely reduced by a smaller dose of GO (1 ng/ml) and significantly reduced by a larger dose (4 ng/ml) up to +19% (Figure 7).

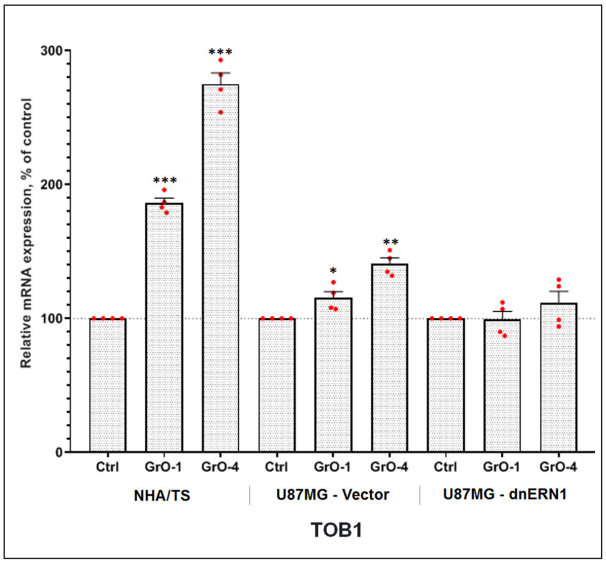

Figure 6. The impact of graphene oxide nanoparticles in doses 1 and 4 ng/ml (GrO-1 and GrO-4, respectively) for 24 h on the expression of TOB1 mRNA in normal human astrocytes line NHA/TS (normal astrocytes) and U87MG glioblastoma cells with native ERN1 (glioblastoma cells) and knockdown ERN1 (ERN1 knockdown glioblastoma cells). Values of this mRNA expression were normalized to ACTB mRNA expression. Ctrl – control cells. Data are presented as mean±SEM (n=4). *p<0.05; **p<0.01; ***p<0.001 vs. corresponding control.

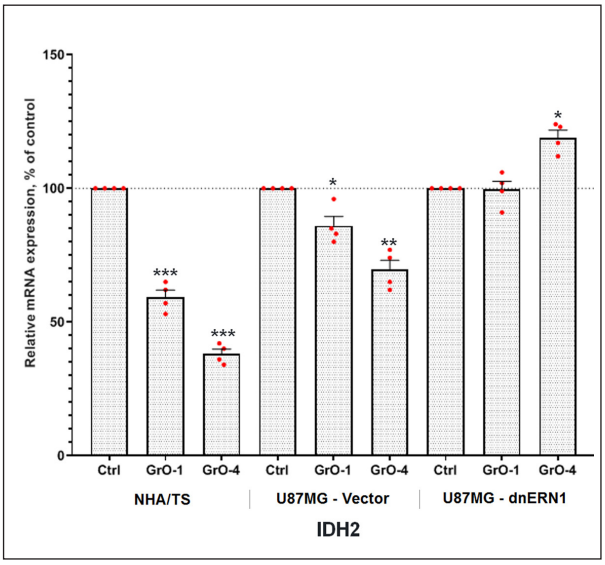

Figure 7. The impact of graphene oxide nanoparticles in doses 1 and 4 ng/ml (GrO-1 and GrO-4, correspondingly) for 24 h on the expression of IDH2 mRNA in normal human astrocytes line NHA/TS (normal astrocytes) and U87MG glioblastoma cells with native ERN1 (glioblastoma cells) and knockdown ERN1 (ERN1 knockdown glioblastoma cells). Values of this mRNA expression were normalized to ACTB mRNA expression. Ctrl – control cells. Data are presented as mean±SEM (n=4). *p<0.05; **p<0.01; ***p<0.001 vs. corresponding control.

Discussion

The results of this investigation demonstrated that nGO 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 NPs (Figure 8).

The expression of *DNAJB9* gene in normal human astrocytes is more sensitive to the effect of relatively small doses of nGO than in glioblastoma cells and the changes in this gene expression introduced by nGO are mediated by the ER stress. Protein DNAJB9 represents the ER luminal co-chaperone ERdj4, which is induced by ER stress and is involved in signaling pathways of this stress. It selectively represses ERN1 by forming a complex between the ER chaperone HSPA5/BiP and the luminal stress-sensing domain of ERN1 (Amin-Wetzel et al. 2017). DNAJB9 plays an important role in protecting stressed cells from apoptosis through inhibition pro-apoptotic function of p53 (Lee et al. 2015). It agrees well with numerous data regarding the role of ER stress in the mechanism of genotoxic action of NPs with different composition and structure (Chen et al. 2014; Barberet et al. 2017; Cao et al. 2017; Simon et al. 2017; Hu et al. 2018; Liu et al. 2022; Minchenko et al. 2023).

Furthermore, the upregulation of *EDEM1* gene expression introduced by relatively small doses of nGO is mediated by the ER stress. Protein encoding by *EDEM1* gene represents an important quality control factor directly involved in the ER-associated degradation of misfolded proteins to maintain homeostasis, and alteration of its functions enhance ATF6 pro-survival signaling (Papaioannou et al. 2018; Chiritoiu et al. 2020). Moreover, a somatic variant of EDEM1 that alters ATF6 signaling has been found in hepatocellular carcinoma (Papaioannou et al. 2018). Thus, the expression of *EDEM1* gene is more resistant to toxic action of nGO NPs in glioblastoma cells with native ERN1 than normal human astrocytes possibly due to ER stress-mediated polyresistance of cancer cells (Minchenko et al. 2021).

The results of this study also demonstrate that the upregulation of transcription factor *DDIT3*, *ATF3*, and *ATF4* gene expressions introduced by relatively small doses of nGO are also mediated by the ER stress and changes in the expression of these genes were much stronger in normal than malignant cells. Furthermore, the upregulation of *TOB1* and downregulation of the *IDH2* gene expressions introduced by relatively small doses of nGO are also mediated by the ER stress and the change in these gene expressions were more pronounced in normal astrocytes than in glioblastoma cells.

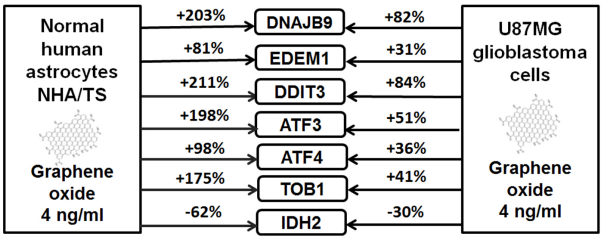

Figure 8. Schematic demonstration of the impact of graphene oxide nanoparticles in a dose of 4 ng/ml for 24 h on the expression of DNAJB9, EDEM1, DDIT3, ATF3, ATF4, TOB1, and IDH2 mRNAs in normal human astrocytes line NHA/TS and U87MG glioblastoma cells with native ERN1.

It is important to note that this stress is permanent because nGO NPs are not biodegradable and can mediate prolonged genome reprogramming and genotoxicity by disrupting the functional integrity of the genome. The ER stress may have a central role in the impacts of nGO on cell metabolism, survival, and cancerogenesis. However, precise mechanisms of GO NP genotoxicity are still largely unknown and warrant further investigation.

Conclusions

We present that the nGO, which can potentially be used in the biomedical devices including cancer therapy, exerts 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 possibly due to ER stress-mediated polyresistance of cancer cells and reflects a genotoxic impact of these NPs through ER stress. Currently, very little is known about the genotoxicity and neurotoxicity of nGO as well as many other NPs. To better understand the detailed molecular mechanisms of their action, it is of importance, not only in the frame of promising therapeutic applications of carbon nanomaterials, but also is required for careful assessment of potential exposure-related risks for human health. The results of this study indicate that there is a need for further in-depth investigations of graphene-based NPs, not only on tumor cells as targets, but primarily on various normal cells of the body to prevent the side effects of nanotherapy. These data also suggest that more caution is needed in biomedical applications of the carbon NPs.

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