

THE MULTIFACETED ROLE OF ENDOPLASMIC RETICULUM STRESS IN THE REGULATION OF GENE EXPRESSION IN NORMAL ASTROCYTES AND GLIOBLASTOMA CELLS

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Aim. To determine the role of the endoplasmic reticulum (ER) in regulating gene expression in normal astrocytes and glioblastoma cells under the application of carbon nanoparticles as anti-tumor agents and inhibition of ERN1 (endoplasmic reticulum to nucleus signaling 1).

Methods. Aqueous dispersions of graphene oxide (GO) and aqueous suspensions of single-walled carbon nanotubes (SWCNTs) were used to study the impact of carbon nanoparticles. Experiments were conducted on regular human astrocytes line NHA/TS and U87MG glioblastoma cells. Two sublines of ERN1 knockdown cells were applied to determine the role of ERN1 in the control of gene expression. Quantitative RT-PCR detected the expression level of mRNA and microRNA.

Results. It was shown that GO and SWCNTs affect the expression of numerous regulatory genes in both normal human astrocytes and glioblastoma cells, but the effect was less pronounced in glioblastoma cells than in normal astrocytes and preferentially mediated by ERN1. The expression of serine biosynthesis genes is controlled by ERN1 protein kinase or endoribonuclease activity. Furthermore, the inhibition of functional activity of ERN1 modifies the effects of hypoxia on gene expression.

Conclusions. Thus, these results demonstrated that ER stress plays an essential role in controlling the expression of various regulatory genes in glioblastoma cells and that normal astrocytes are more sensitive to the effect of carbon nanoparticles than glioblastoma cells.

Keywords: ER stress, carbon nanoparticles, mRNA and microRNA expression, DNAJB9, serine synthesis genes, glioblastoma cells, normal astrocytes.

Endoplasmic reticulum (ER) stress is an essential factor in the development of many pathological conditions, including the growth of malignant tumors, as well as various complications due to the action of nanoparticles. Previous studies have shown that inhibition of ERN1 (endoplasmic reticulum to nucleus signaling 1), a key sensor in the ER stress response, suppressed glioma cell proliferation and decreased the effect of nanoparticles. The exact mechanisms of this influence remain poorly understood, but a possible pathway for their action may involve the recruitment of both protein kinase and endoribonuclease activity of ERN1 signaling protein. Moreover, ER stress is responsible for nanoparticle toxicity as well as for regulation of the expression of pro-oncogenic gene expression.

Aim. To investigate the comparative effects of carbon nanoparticles on the expression of key regulatory genes in normal astrocytes and glioblastoma cells and find out the molecular mechanism of regulation of serine synthesis gene expression in glioblastoma cells with different ERN1 knockdowns.

Methods. Experiments were conducted on U87MG glioblastoma cells and their sublines with different ERN1 knockdown and regular human astrocytes line NHA/TS. Aqueous dispersions of

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graphene oxide (GO) and aqueous suspensions of single-walled carbon nanotubes (SWCNTs) were used to study the impact of carbon nanoparticles. The measurement of mRNA and microRNA expression levels was carried out using quantitative real-time polymerase chain reaction.

Results and Discussion. Our studies indicate that even small doses of graphene oxide (1 and 4 ng/mL) and SWCNTs (2 and 8 ng/mL) disrupt the expression of numerous regulatory genes in regular human astrocytes line NHA/TS [1–4]. Thus, the treatment of normal human astrocytes with GO nanoparticles in a dose of 1 ng/ml of medium) introduced a substantial increase in *DNAJB9* (heat shock protein family member B9 DnaJ) gene expression (by 126%, $P < 0.001$) as compared to the control [1]. A much stronger effect on the expression of the *DNAJB9* gene (203%, $P < 0.001$) was observed when using a bigger dose (1 ng/ml of medium) of these nanoparticles [1].

At the same time, the effect of GO nanoparticles on the expression of this gene was significantly lower (+41%, $P < 0.01$, and +82%, $P < 0.001$, respectively, for 1 and 4 ng/ml of nanoparticles) in glioblastoma cells with native ERN1 (Figure). It is interesting to note that the knocking down of ERN1 signaling protein in U87MG glioblastoma cells reduced the effect of these nanoparticles on *DNAJB9* gene expression close to a control level [1]. Similar results were shown for genes encoded transcription factors ATF3 and ATF4 [1]. These results demonstrated that the expression of different genes in normal astrocytes is more sensitive to GO nanoparticles than glioblastoma cells and that the effect of these nanoparticles is mainly mediated by ERN1 signaling protein, a major signaling pathway of ER stress. Thus, the impact of GO nanoparticles on different gene expressions is dose-dependent and gene-specific in both normal astrocytes and glioblastoma cells. Moreover, the gene specificity of the action of these nanoparticles is determined mainly by ER stress [1]. These carbon nanoparticles also affect microRNA expression levels, which have binding sites with target mRNAs, indicating the presence of post-transcriptional regulatory mechanisms for these genes [2].

It was also found that carbon nanotubes affect the expression of numerous genes in normal human astrocytes and glioblastoma cells. It was shown a more pronounced increase in the expression level of *BRCA1* (breast cancer type 1 susceptibility protein) and *DNAJB9* in normal cells [4]. Suppression of ERN1 signaling protein in glioblastoma cells decreased the effect of SWCNTs, thereby confirming the role of ER stress in affecting these gene expressions by carbon nanoparticles [4]. Furthermore, the treatment of normal human astrocytes with carbon nanotubes significantly affects the expression level of numerous microRNAs, which have binding sites with target mRNAs, indicating the possibility of a post-transcriptional mechanism for the regulation of studied gene expressions [3].

It has been shown that the expression level of genes responsible for serine synthesis was generally decreased in glioblastoma cells with inhibited both enzymatic activities of the ERN1, except *SHMT1* (serine hydroxymethyltransferase 1) gene, whose expression was sharply increased [5]. At the same time, the suppression of ERN1 endoribonuclease activity affects the expression of the *ATF4* (activating transcription factor 4) gene but does not significantly change the expression of *PHGDH* (phosphoglycerate dehydrogenase), *SHMT1*, and *SHMT2* genes. These results demonstrate that the expression of the *ATF4* gene is controlled by ERN1 endoribonuclease activity and that the expression of *PHGDH*, *SHMT1*, and *SHMT2* genes is regulated by ERN1 protein kinase activity [5]. It was also shown that silencing of XBP1 (X-box binding protein 1) by specific siRNA also suppresses only *ATF4* gene expression in glioblastoma cells. Still, silencing of ERN1 by specific siRNA decreases the expression of *PHGDH*, *SHMT2*, and *ATF4* genes. silencing of ERN1 by specific siRNA decreases the expression of *PHGDH*, *SHMT2*, and *ATF4* genes. At the same time, silencing of ERN1 strongly increases the expression of *SHMT1* [5].

The inhibition of ERN1 signaling protein also affects the expression of several microRNA expressions, which is responsible for post-transcriptional control of serine synthesis mRNA expressions in glioblastoma cells and possibly contributes to the suppression of these cell proliferation [5].

The significant results of analyzed data concerning the multifaceted role of ER stress in the regulation of gene expression in normal astrocytes and glioblastoma cells are summarized in Fig.

Conclusions. Changes in gene expression induced by graphene oxide nanoparticles and single-walled carbon nanotubes are mainly associated with the induction of ER stress and alterations in microRNA expression, which are key regulators of ER homeostasis and critical players in UPR (unfolded protein response) signaling. These changes are gene-specific and depend on ER stress because the inhibition of ERN1 significantly modifies the effects of carbon nanoparticles on the expression of studied genes. Meanwhile, normal human astrocytes are more sensitive to both graphene oxide nanoparticles and SWCNTs exposure compared to glioblastoma cells in a dose-

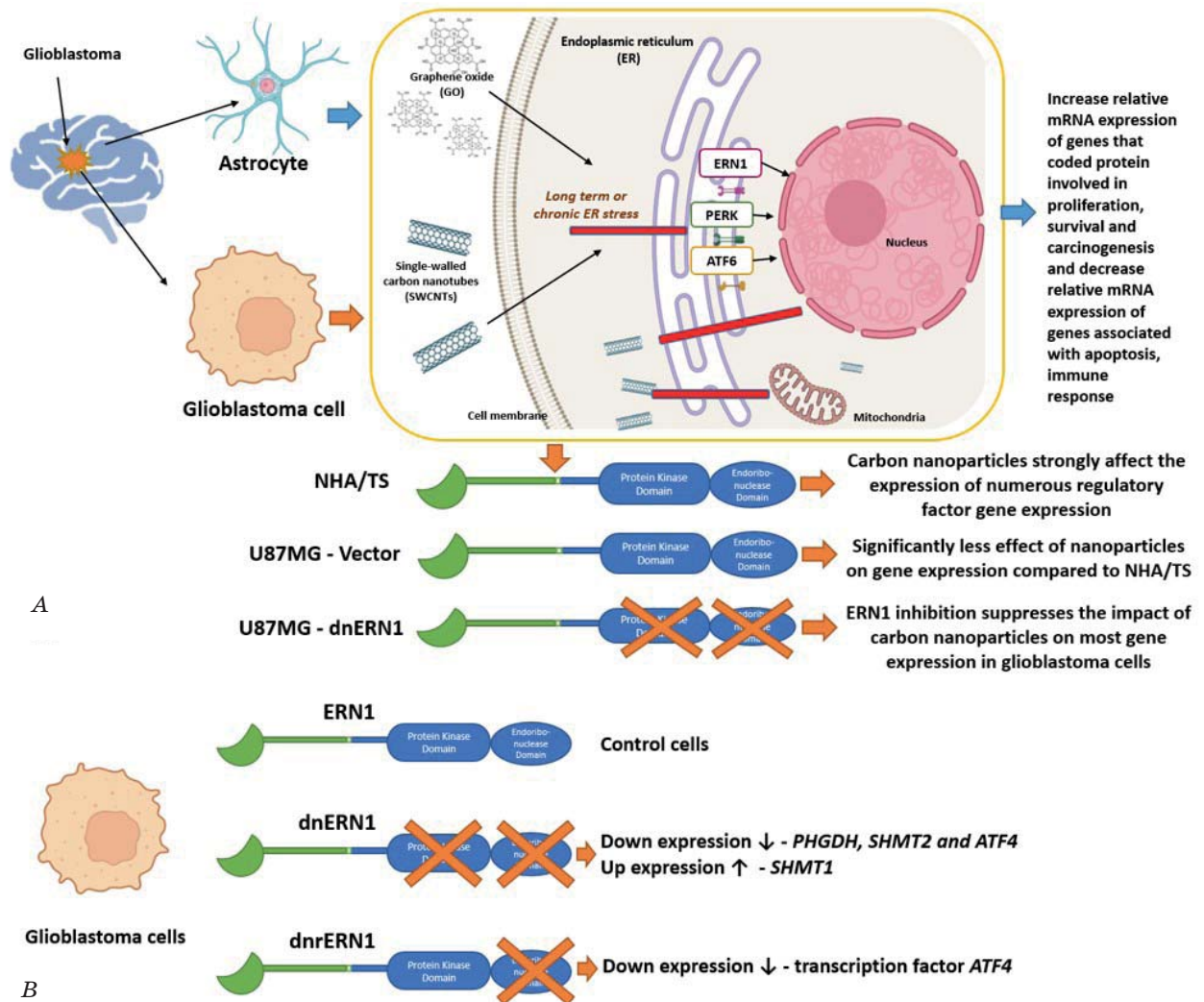


Figure. A — Effect of ERN1 enzymatic activities on the expression level of genes in U87MG glioblastoma cells with suppressed both enzymatic activities (endoribonuclease and protein kinase) of ERN1 (dnERN1) and in cells without only endoribonuclease activity of ERN1 (dnrERN1) compared to control cells transfected with “empty” vector; **B** — Schematic representation of the effect of carbon nanoparticles on the expression level of genes associated with proliferation and survival processes in normal human astrocytes line NHA/TS, U87 glioblastoma cells transfected with a vector (U87-Vector) and cells with a dominant-negative ERN1 construct (U87-dnERN1)

dependent manner, which is likely due to the tumor cell resistance to various external factors. This serves as a cautionary point regarding the biomedical applications of carbon nanoparticles. It has been established that the protein kinase activity of ERN1 is an essential regulator of serine biosynthesis gene expression, such as *PHGDH*, *SHMT1*, and *SHMT2* genes. At the same time, the ERN1 endoribonuclease activity controls the level of *ATF4* gene expression.

Authors' Contribution

The authors contributed to the experimental work equally.

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