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GRAPHENE OXIDE AFFECT THE EXPRESSION OF PROLIFERATION RELATED GENES AND MICRORNA IN NORMAL HUMAN ASTROCYTES

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Graphene and its oxide are nanomaterials, which have the exceptional physicochemical properties considered currently to be very promising because of their great potential applications in various industries. Graphene oxide, an oxidation derivative of graphene, is considered as one of the nanomaterials attractive for biomedical applications in the fields of drug delivery, cancer therapy, and diagnostics [1–3]. The increasing exploitation of graphene-based materials requires a comprehensive evaluation of the potential impact of these unique carbon nanoparticles on human health and the environment [4]. At the same time, potential risks have also been recognized, as the hazardous impact of various carbon nanoparticles on human health and the environment was previously shown [5–6]. Thus, the toxic potential of carbon nanoparticles was reported in numerous cell lines and animal models and their long-term toxicity has attracted increasing concern.

Aim. In this study we investigate the impact of low doses of graphene oxide on the expression of key regulatory genes which control cell proliferation as well as microRNAs in normal human astrocytes.

Methods. 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 [7]. These astrocytes were treated with graphene oxide (1 and 4 ng/ml of medium) for 24 hrs. Graphene oxide (2 mg/ml, dispersion in water) was received from Sigma-Aldrich Chemie GmbH, Germany. Total RNA was extracted using TRIZOL reagent. For reverse transcription of mRNAs we used Thermo Scientific Verso cDNA Synthesis Kit (Germany). The values of mRNA expressions were normalized to the level of ACTB mRNA and represented as percent of control (100%). For polyadenylation and reverse transcription of miRNAs we used Mir-X miRNA First-Strand Synthesis Kit (Takara, Japan). The expression level of microRNAs was studied by real-time qPCR using SYBRGreen Mix and specific for each miRNA forward primers and universal reverse primer. For normalization of microRNA expressions the level of U6 RNA expression was used.

Results. It was shown that the expression level of *TOB1, HSPA5, EDEM1, MYBL1*, and *MYBL2* significantly increased in normal human astrocytes line NHA/TS, which were treated with graphene oxide (1 and 4 ng/ml of medium) for 24 hrs. Up-regulation of these genes expression was dose-dependent: bigger dose of graphene oxide (4 ng/ml of medium) introduced more significant changes in the expression of all these genes. Furthermore, bioinformatics analysis of 3′-untranslated regions of mRNA allowed identifying binding sites of microRNA: miR-19a for MYBL1, miR-143 for MYBL2 and miR-182 for TOB1. It was also shown that the expression of all these microRNA significantly down-regulated by graphene oxide, supporting the idea of both post-transcriptional and transcriptional regulation of *MYBL1, MYBL2* and *TOB1* gene expressions (Figure).

Discussion. Results of this study showed that graphene oxide affects the expression of *MYBL1, MYBL2,* and *TOB1* genes through both transcriptional and post-transcriptional mechanisms. It is possible that these changes in gene expressions are mediated by the endoplasmic reticulum stress, which strongly induces *HSPA5/GRP78* gene expression.

Conclusions. Graphene oxide significantly disturbs genome stability by up-regulation of the expression of key regulatory genes and down-regulation of microRNA.

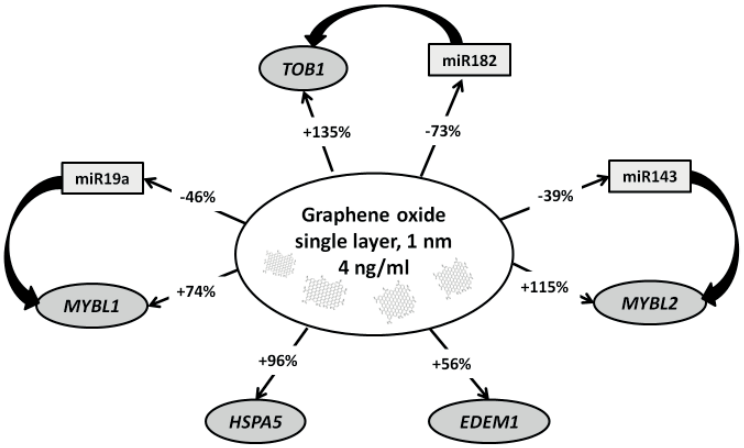

Fig. **The Impact of low dose of graphene oxide on the expression level of proliferation related genes and microRNAs in normal human astrocytes**

Key words: Graphene oxide, proliferation, gene expression, microRNA, normal human astrocytes.

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