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Expression of DNAJB9 and some other genes is more sensitive to SWCNTs in normal human astrocytes than glioblastoma cells

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Objective. Single-walled carbon nanotubes (SWCNTs) are considered to be one of the nanomaterials attractive for biomedical applications, particularly in the health sciences as imaging probes and drug carriers, especially in the field of cancer therapy. The increasing exploitation of nanotubes necessitates a comprehensive evaluation of the potential impact of these nanomaterials, which purposefully accumulate in the cell nucleus, on the human health and the function of the genome in the normal and tumor tissues. The aim of this study was to investigate the sensitivity of the expression of *DNAJB9* and some other genes associated with the endoplasmic reticulum (ER) stress and cell proliferation to low doses of SWCNTs in normal human astrocytes (NHA/TS) and glioblastoma cells (U87MG) with and without an inhibition of ERN1 signaling pathway of the ER stress.

Methods. Normal human astrocytes, line NHA/TS and U87 glioblastoma cells stable transfected by empty vector or dnERN1 (dominant-negative construct of ERN1) were exposed to low doses of SWCNTs (2 and 8 ng/ml) for 24 h. RNA was extracted from the cells and used for cDNA synthesis. The expression levels of DNAJB9, TOB1, BRCA1, DDX58, TFPI2, CLU, and P4HA2 mRNAs were measured by a quantitative polymerase chain reaction and normalized to ACTB mRNA.

Results. It was found that the low doses of SWCNTs up-regulated the expression of *DNAJB9*, *TOB1*, *BRCA1*, *DDX58*, *TFPI2*, *CLU*, and *P4HA2* genes in normal human astrocytes in dose-dependent (2 and 8 ng/ml) and gene-specific manner. These nanotubes also increased the expression of most studied genes in control (transfected by empty vector) U87 glioblastoma cells, but with much lesser extent than in NHA/TS. However, the expression of *CLU* gene in control U87 glioblastoma cells treated with SWCNTs was down-regulated in a dose-dependent manner. Furthermore, the expression of *TOB1* and *P4HA2* genes did not significantly change in these glioblastoma cells treated by lower dose of SWCNTs only. At the same time, inhibition of ERN1 signaling pathway of ER stress in U87 glioblastoma cells led mainly to a stronger resistance of *DNAJB9*, *TOB1*, *BRCA1*, *DDX58*, *TFPI2*, and *P4HA2* gene expression to both doses of SWCNTs.

Conclusion. The data obtained demonstrate that the low doses of SWCNTs disturbed the genome functions by changing the levels of key regulatory gene expressions in gene-specific and dose-dependent manner, but their impact was much stronger in the normal human astrocytes in comparison with the tumor cells. It is possible that ER stress, which is constantly present in tumor cells and responsible for multiple resistances, also created a partial resistance to the SWCNTs action. Low doses of SWCNTs induced more pronounced changes in the expression of diverse genes in the normal human astrocytes compared to glioblastoma cells indicating for a possible both genotoxic and neurotoxic effects with a greater extent in the normal cells.

Key words: SWCNTs, DNAJB9, BRCA1, DDX58, mRNA expression, ERN1, normal human astrocytes, U87 glioblastoma cells

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Single-walled carbon nanotubes (SWCNTs) have exceptional physiochemical properties and are currently considered to be one of the nanomaterials attractive for the health sciences as imaging probes and drug carriers, especially in the field of the cancer therapy (Avitabile et al. 2018; Eldawud et al. 2018; Yan et al. 2019). The increasing exploitation of unique carbon nanoparticles requires a comprehensive evaluation of their potential impact on the environment and the human health (Yan et al. 2019; Chen et al. 2020; Li et al. 2021; Rudnytska et al. 2022). The hazardous impact of various carbon nanotubes on the environment and the mammalian normal and cancer cells has also been recognized (Francis and Devasena 2018; Kavosi et al. 2018; Minchenko et al. 2018; Yan et al. 2019; Jiang et al. 2020; Rudnytska et al. 2021). Actually, the toxic potential of carbon nanoparticles has been reported in numerous cell lines and animal models and their long-term toxicity has led to an increasing concern (Ahmadi et al. 2017; Eldawud et al. 2018; Minchenko et al. 2022).

It has also been shown that various carbon nanotubes are able to penetrate over the blood-brain barrier and accumulate in the nucleus, which purposefully allows their use as imaging probes and drug carriers in cancer therapy (Aragon et al. 2017). However, carbon nanotubes are not biodegradable, which consequently restricts their use in medicine and biology since the raising concerns of its chronic genome toxicity (Ahmadi et al. 2017; Jiang et al. 2020). Previously, it was shown that carbon nanoparticles may interfere with an immune response, because of their strong suppressing effect on the expression of cell surface glycoproteins, such as HLA-DRA (major histocompatibility complex, class II, DR alpha) and HLA-DRB1 (Minchenko et al. 2018). Furthermore, SWCNTs disturbed the genome stability through dysregulation of lamin B expression showing the genotoxic effect of these nanoparticles (Minchenko et al. 2018).

Recently, we have also shown that SWCNTs dysregulated the expression of numerous genes in normal human astrocytes (Rudnytska et al. 2021). These genes are associated with endoplasmic reticulum (ER) stress and cell proliferation, cell survival, and cancerogenesis. At the same time, detailed mechanisms of the toxicity of this type of carbon nanoparticles have not been still fully revealed. One of their possible mechanisms of toxicity may include signaling pathways of the ER stress, as it has been shown for different nanoparticles (Boran and Ulutas 2016; Cao et al. 2017; Simon et al. 2017; Chen et al. 2020). There are data demonstrating that modulation of the ER stress by chemicals may alter the toxicity of some nanoparticles (Simon et al. 2017). Chemical compound 4-phenylbutyric acid repressed most effects of nanoparticles, particularly the titanium dioxide nanoparticles, through the relief of some aspects of the ER stress signaling (Simon et al. 2017; Hu et al. 2018). However, it is very important to note that understanding of the molecular mechanism, by which the carbon nanoparticles may induce toxicity, is significant for both nanotoxicology and nanomedicine spheres.

For the present study, several genes encoding the key regulatory proteins such as DnaJ heat shock protein family (Hsp40) member B9 (DNAJB9), DExD/H-box helicase 58 (DDX58), breast cancer 1, early onset (BRCA1), clusterin (CLU), transducer of ERBB2, 1 (TOB1), tissue factor pathway inhibitor 2 (TFPI2), and prolyl 4-hydroxylase alpha polypeptide II (P4HA2) were selected. The DNAJB9 protein, also known as ER DnaJ homolog 4 (ERdj4), is member of Hsp40 family of heat shock proteins, functions in many cellular processes by regulating the ATPase activity of 70 kDa heat shock proteins and involved in stress response (Amin-Wetzel et al. 2017). There are also data indicating that DNAJB9 also participate in chemotherapy resistance and metastasis (Wang et al. 2020; Kim et al. 2021). The DDX58 protein, also known as retinoic acid-inducible gene 1 protein (RIG1), mediates the signal transduction events playing a role in the regulation of cell development, growth and motility and has a relation to cancerogenesis and the regulation of immune response (Piras et al. 2022; Kouwaki et al. 2023). Protein BRCA1 is a nuclear phosphoprotein, which represents a regulatory subunit 53 of protein phosphatase 1 that plays an important role in maintaining the genomic integrity and stability and it has the E3 ubiquitin-protein ligase activity, which is required for its tumor suppressor function (Jhanwar-Uniyal 2003; Takaoka and Miki 2018; Tahir et al. 2020) and it is also required for the normal cell cycle progression from G2 to mitosis. Clusterin functions as extracellular chaperone that prevents aggregation of nonnative proteins and involved in several basic biological events such as cell death and tumor progression (Liu et al. 2018; Patarat et al. 2021). The TOB1 protein has the potential to regulate cell growth and functions as a putative tumor suppressor in cancer cells (Bai et al. 2020; Zhao et al. 2021). The TFPI2 inhibits a variety of serine proteases and has been identified as a tumor suppressor gene in several types of cancer (Sierko et al. 2007; Zhao et al. 2020). It has an important role in the negative control of cancer cell proliferation and invasion through regulation of ERK signaling

and other pathways (Wang et al. 2018; Zhao et al. 2020). The prolyl 4-hydroxylase is a key enzyme in collagen synthesis and deposition. In collagen and related proteins, the prolyl 4-hydroxylase catalyzes the formation of 4-hydroxyproline that is essential to the regulation of metabolism through post-translational modification of the human proteome and is also required for the tumor growth and metastasis (Wang et al. 2019; Cao et al. 2020).

Since SWCNTs are currently considered to be one of the nanomaterials attractive for the health sciences as drug carriers and cancer therapy, we decided to compare the effect of these nanoparticles on normal and tumor cells. Furthermore, different nanoparticles are toxic and their toxicity maybe mediated by signaling pathways of the ER stress, including ERN1 (endoplasmic reticulum to nucleus signaling 1) sensor-signaling pathway (Boran and Ulutas 2016; Cao et al. 2017; Simon et al. 2017; Hu et al. 2018; Chen et al. 2020). In this regard, we also decided to conduct research on gene expression level using glioblastoma cells with ERN1 knockdown. Thus, this study was aimed to reveal the sensitivity of the expression of key regulatory genes such as *DNAJB9*, *DDX58*, *BRCA1*, *TFPI2*, *CLU*, *TOB1*, and *P4HA2* to low doses of SWCNTs in normal human astrocytes line NHA/TS as well as U87 glioblastoma cells with and without of ERN1 inhibition.

Materials and Methods

Cell culture and treatment. The immortalized normal human astrocytes (NHA/TS cell line) were received from Dr. K. Sasai and Dr. S. Tanaka (Japan) and left grown at 37°C under a humidified atmosphere of 95% air and 5% CO_2 as described by Sasai et al. (2007). The sublines of U87MG glioblastoma cells and their growing were described previously (Auf et al. 2013). These cell lines were treated with SWCNTs (2 and 8 ng/ml) for 24 h. The functionalized SWCNTs were received from Prof. Uwe Ritter, Institut fur Chemie und Biotechnik, Technische Universitat Ilmenau, Ilmenau, Germany, and their characteristics were described previously (Minchenko et al. 2022).

RNA extraction and cDNA synthesis. Total RNA was extracted from NHA/TS cells using TRIZOL reagent as described previously (Auf et al. 2013; Rudnytska et al. 2021). 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 pellets were dissolved again in nuclease-free water. RNA quantity as well as spectral characteristics was measured using NanoDrop One (Thermo Scientific). Thermo Scientific Versco cDNA Synthesis Kit was used for the mRNA reverse transcription.

Real-time quantitative polymerase chain reaction. The expression levels of DNAJB9, DDX58, BRCA1, CLU, TOB1, TFPI2, and P4HA2 mRNAs were measured by real-time qPCR using "QuantStudio 5 Real-Time PCR System" (Applied Biosystems) using Luna Universal qPCR Master Mix, New England BioLabs, Ipswich, MA, USA. For analysis of mRNA expressions, four different RNA samples for each experimental group as well as pairs of the specific primers (Table 1) received from Sigma-Aldrich

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 (ERdj4)	DnaJ heat shock protein family (Hsp40) member B9 (endoplasmic reticulum DnaJ homolog 4)	F: 5'- gtcggagggtgcaggatatt R: 5'- tcagggtggtacttcatggc	217–236 407–388	NM_012328.3
DDX58 (RIG1)	DExD/H-box helicase 58 (retinoic acid-inducible gene 1 protein)	F: 5'- gcaacagctgagaatgtccc R: 5'- ggcctgaagatcctccaagt	1009–1028 1231–1212	NM_014314.4
CLU	Clusterin	F: 5'- tcaaaatgctgtcaacgggg R: 5'- tggtctcattgcacactcct	213–232 391–372	NM_001831.4
BRCA1 (PPP1R53)	Breast cancer 1, early onset (protein phosphatase 1, regulatory subunit 53)	F: 5'- tgaagaaagaggaacgggct R: 5'- tggctcccatgctgttctaa	4145–4164 4384–4365	NM_007294.4
TFPI2	Tissue factor pathway inhibitor 2	F: 5'- tctgccaatgtgactcgcta R: 5'- ccggattctactggcaaagc	577–596 759–740	NM_006528.4
TOB1	Transducer of ERBB2, 1	F: 5'- agcccgaacaagatcactca R: 5'- cacgtctcctgggaagctta	293–312 516–535	NM_005749.4
P4HA2	Prolyl 4-hydroxylase alpha polypeptide II	F: 5'- gggtttccaaaagctcctgg R: 5'- ttccccgtccctaaatgctt	1552–1571 1759–1740	NM_005749.4
ACTB	beta-actin	F: 5'- catccgcaaagacctgtacg R: 5'- cctgcttgctgatccacatc	948–967 1165–1146	NM_001101.5

(St. Louis, MO, USA) were used. Polymerase chain reaction was performed in triplicates. The quality of amplification products was analyzed by melting curves and electrophoresis in 3% agarose gel and visualized by SYBR* Safe DNA Gel Stain (Life Technologies, Carlbad, CA, USA). Quantitative PCR analysis was performed using a special computer program "Differential Expression Calculator".

Statistical analysis. All values are expressed as mean ± SEM obtained from triplicate measurements performed in 4 independent experiments. Statistical analysis was performed according to Student's *t*-test using Excel program as described previously (Rudnytska et al. 2021). A value of $p<0.05$ was considered statistically significant.

Results

We investigated the impact of SWCNTs on the genome function in normal human astrocytes and U87MG glioblastoma cells by measuring the expression level of mRNAs, which encode important regulatory proteins related to the control of cell proliferation, metabolism, and cancerogenesis. As shown in Figure 1, the exposure of normal human astrocytes line NHA/TS to 2 different doses of SWCNTs (2 and 8 ng/ml for 24 h) led to a significant up-regulation of the expression of DNAJB9 mRNA (+102 and +160%, respectively). At the same time, the effect of these nanotubes on DNAJB9 mRNA expression in glioblastoma cells was significantly lesser: +55 and +96%, respectively for 2 and 8 ng/ml of SWCNTs (Figure 1). However, in glioblastoma cells with ERN1 knockdown inducible effect of SWCNTs on the expression of DNAJB9 mRNA did not show at all (Figure 1). Furthermore, treatment of these cells by higher dose of SWCNTs (8 ng/ml for 24 h) even slightly suppressed the expression of DNAJB9 mRNA (-17%). Stronger impact of SWCNTs was shown for DDX58/RIG1 mRNA expression in normal human astrocytes. Thus, as shown in Figure 2, treatment of normal human astrocytes with SWCNTs (2 and 8 ng/ml for 24 h) led to a strong up-regulation of *DDX58* gene expression (+165 and +272%, respectively), but in glioblastoma cells the impact of these nanoparticles was significantly smaller: +52 and +126%, respectively for 2 and 8 ng/ml of SWCNTs. At the same time, the knockdown of ERN1 signaling

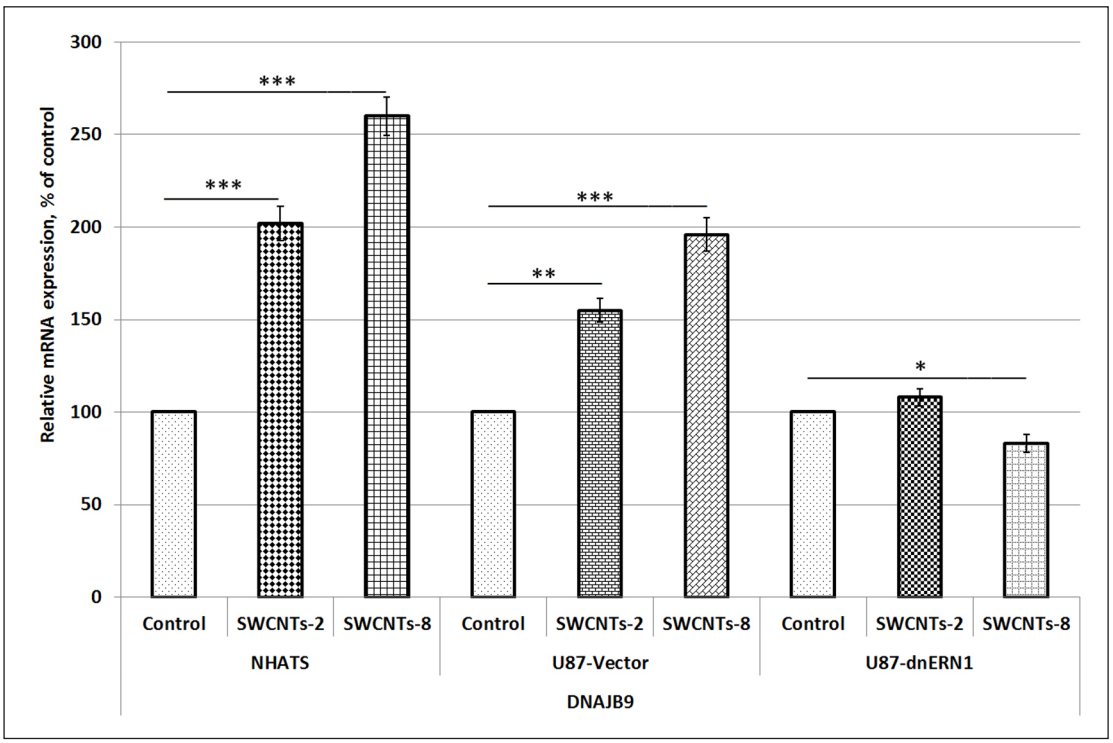


Figure 1. The impact of SWCNTs (2 and 8 ng/ml, for 24 h) on the DnaJ heat shock protein family (Hsp40) member B9 (DNAJB9), known also as an endoplasmic reticulum DnaJ homolog 4 (ERdj4), mRNA expression in normal human astrocytes line NHA/TS and U87 glioblastoma cells stable transfected by empty vector (U87-Vector) or dnERN1 for ERN1 knockdown (U87-dnERN1) versus not treated (control) cells. Values of this mRNA expression were normalized to the ACTB mRNA level; n=4.

in U87 glioblastoma cells led to complete resistance of *DDX58* gene expression to SWCNTs treatment by both doses (Figure 2).

As shown in Figure 3, treatment of normal astrocytes by two different doses of SWCNTs (2 and 8 ng/ml for 24 h) led to a significant up-regulation of *BRCA1* gene expression (+73 and +92%, respectively). Significantly less of an effect of SWCNTs was shown for the expression of BRCA1 mRNA in glioblastoma cells: +16 and +54%, respectively for 2 and 8 ng/ml of these nanoparticles. At the same time, the suppression of ERN1 signaling completely abolished the impact of SWCNTs on BRCA1 mRNA suppression (Figure 3). Furthermore, strong dose-dependent up-regulation was also detected in the expression of TOB1 and TFPI2 mRNAs in the normal human astrocytes 24 h after the SWCNTs treatment (Figures 4, 5). Thus, the expression level of TOB1 mRNA in astrocytes treated with 2 and 8 ng/ml of these carbon nanotubes was increased by +111 and +158%, respectively (Figure 4). At the same time, the impact of SWCNTs exposure on the expression of TFPI2 was also dose-dependent, but less pronounced: at a dose 2 ng/ml increased by 82% and at a higher dose (8 ng/ml) - by 109% (Figure 5). Furthermore, the impact of SWCNTs on TOB1 mRNA expression in glioblastoma cells was observed at higher dose of nanotubes only (+40%), but it was significantly smaller than in normal human astrocytes (Figure 4). At the same time, the expression of TFPI2 was also up-regulated in glioblastoma cells at both doses of SWCNTs but to a much lesser extent (+17 and +65%, respectively for 2 and 8 ng/ml of nanotubes) than in normal astrocytes (Figure 5). The knockdown of ERN1 in glioblastoma cells led to small but statistically significant up-regulation of TOB1 and TFPI2 mRNAs (+16 and +21%, respectively) by bigger dose of SWCNTs only (Figures 4, 5).

Treatment of normal human astrocytes by SWCNTs also led to an up-regulation of the expression of P4HA2 and CLU mRNAs (Figure 6 and 7). Thus, analysis of P4HA2 mRNA expression showed a sharp inducible impact: +107 and +148%, respectively for 2 and 8 ng/ml of these nanoparticles, but for CLU mRNA expression, the effect was significantly smaller: +45 and +94% for 2 and 8 ng/ml of SWCNTs, respectively. The expression of P4HA2 mRNA in glioblastoma cells are more resistant to SWCNTs

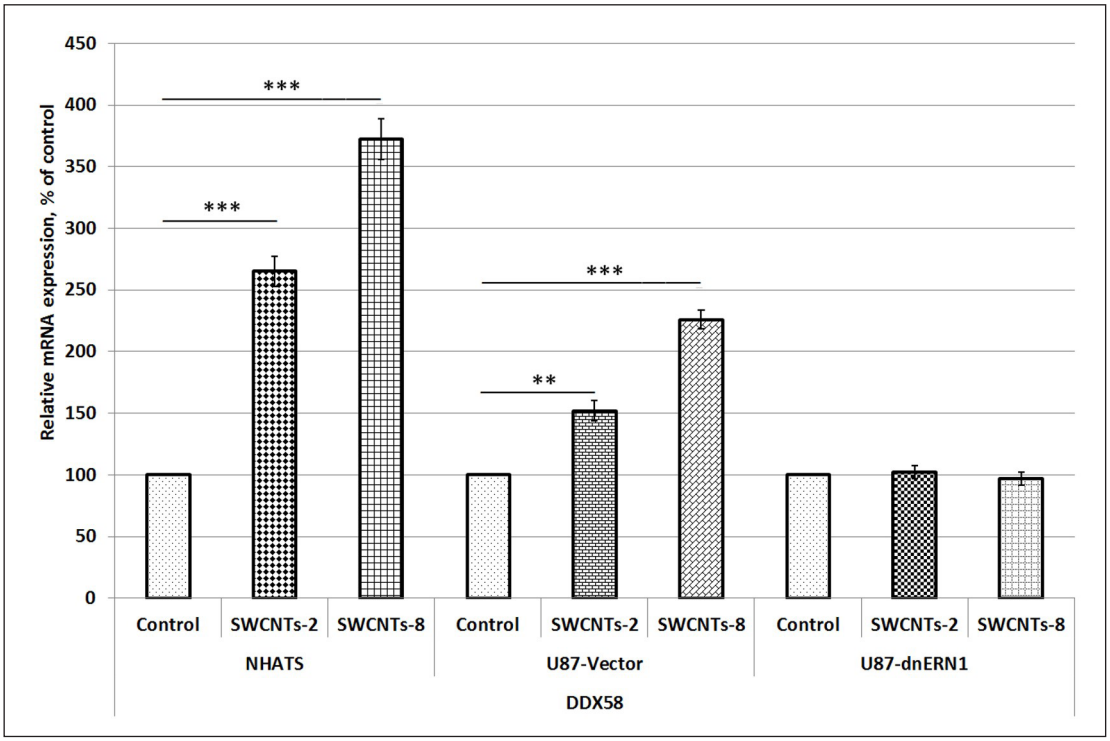


Figure 2. The impact of SWCNTs (2 and 8 ng/ml, for 24 h) on the DExD/H-box helicase 58 (DDX58), known also as a retinoic acid-inducible gene 1 protein (RIG1), mRNA expression in normal human astrocytes line NHA/TS and U87 glioblastoma cells stable transfected by empty vector (U87-Vector) or dnERN1 for ERN1 knockdown (U87-dnERN1) versus not treated (control) cells. Values of this mRNA expression were normalized to the ACTB mRNA level; n=4.

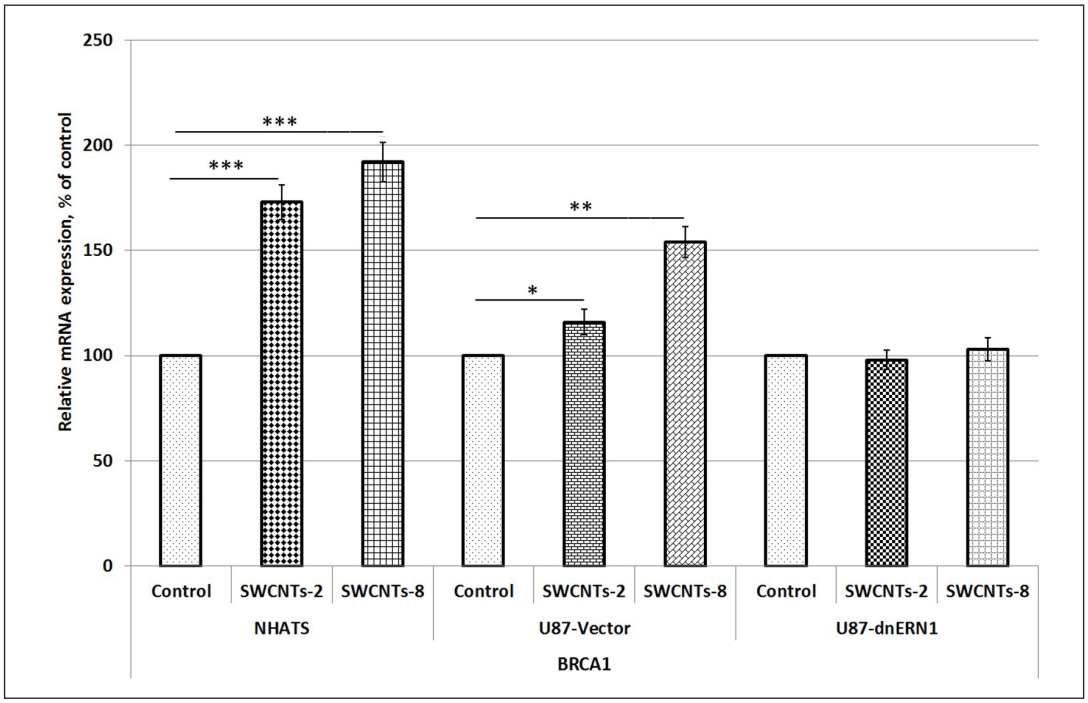


Figure 3. The impact of SWCNTs (2 and 8 ng/ml, for 24 h) on the breast cancer 1, early onset (BRCA1) mRNA expression in normal human astrocytes line NHA/TS and U87 glioblastoma cells stable transfected by empty vector (U87-Vector) or dnERN1 for ERN1 knockdown (U87-dnERN1) versus not treated (control) cells. Values of this mRNA expression were normalized to the ACTB mRNA level; n=4.

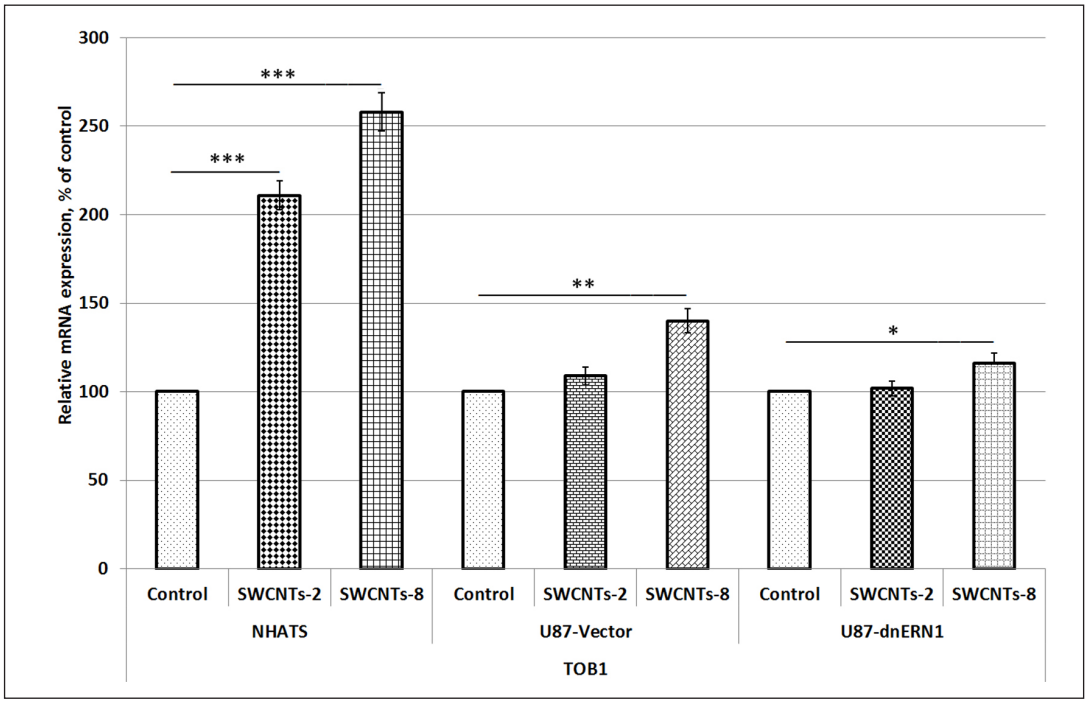


Figure 4. The impact of SWCNTs (2 and 8 ng/ml, for 24 h) on the transducer of ERBB2, 1 (TOB1) mRNA expression in normal human astrocytes line NHA/TS and U87 glioblastoma cells stable transfected by empty vector (U87-Vector) or dnERN1 for ERN1 knockdown (U87-dnERN1) versus not treated (control) cells. Values of this mRNA expression were normalized to the ACTB mRNA level; n=4.

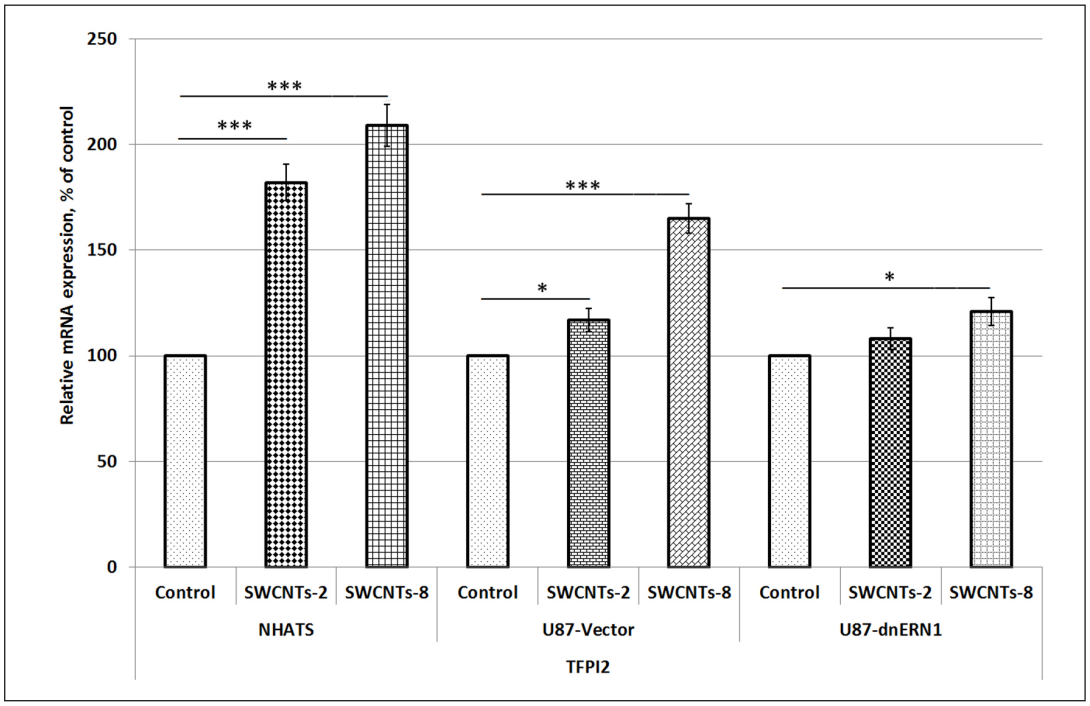


Figure 5. The impact of SWCNTs (2 and 8 ng/ml, for 24 h) on the tissue factor pathway inhibitor 2 (TFPI2) mRNA expression in normal human astrocytes line NHA/TS and U87 glioblastoma cells stable transfected by empty vector (U87-Vector) or dnERN1 for ERN1 knockdown (U87-dnERN1) versus not treated (control) cells. Values of this mRNA expression were normalized to the ACTB mRNA level; n=4.

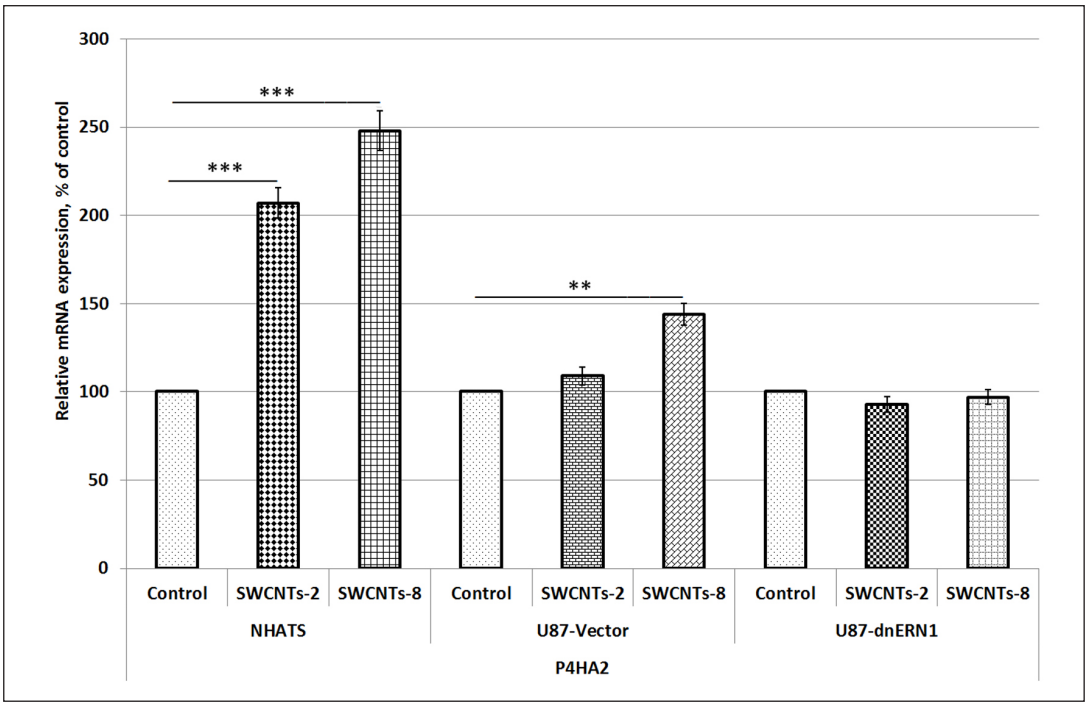


Figure 6. The impact of SWCNTs (2 and 8 ng/ml, for 24 h) on the prolyl 4-hydroxylase alpha polypeptide II (P4HA2) mRNA expression in normal human astrocytes line NHA/TS and U87 glioblastoma cells stable transfected by empty vector (U87-Vector) or dnERN1 for ERN1 knockdown (U87-dnERN1) versus not treated (control) cells. Values of this mRNA expression were normalized to the ACTB mRNA level; n=4.

action and up-regulation of this gene expression is increased (+44%) only in cells treated by higher dose of nanotubes (Figure 6). It is interesting to note that the expression of CLU mRNA is down-regulated in glioblastoma cells (-28 and -41%, respectively for 2 and 8 ng/ml of these nanoparticles), and that ERN1 knockdown did not change these expression levels of CLU mRNA (Figure 7). Therefore, SWCNTs at very low quantities dysregulate the expression of mRNAs, which encode important regulatory enzymes and proteins in normal human astrocytes and glioblastoma cells in a dose-dependent and gene-specific manners but normal astrocytes are more sensitive to genotoxic action of these nanotubes.

Discussion

In this study, we investigated a cell-specific sensitivity of the expression of genes, which encode important regulatory proteins related to the control of metabolism, cell proliferation, and cancerogenesis, to SWCNTs using normal astrocytes and glioblastoma cells. It was found that the exposure of normal human astrocytes and glioblastoma cells to very low quantities of carbon nanoparticles such as SWCNTs significantly alters the expression of DNAJB9, DDX58, BRCA1, CLU, TOB1, TFPI2, and P4HA2 mRNAs, but normal cells were more sensitive to this genotoxic effect of the carbon nanoparticles. These results clearly demonstrate that exposure of normal human astrocytes to SWCNTs dysregulates the genome function that leads to significant dose-dependent and gene-specific changes in the expression of important regulatory factors. The results of this study are in agreement with the data of numerous investigations dealing with the impact of various carbon nanoparticles on genome function and their toxicity (Kavosi et al. 2018; Minchenko et al. 2018, 2022; Yan et al. 2019; Jiang et al. 2020; Rudnytska et al. 2021, 2022).

Although there are data on the toxicity of carbon nanotubes as anti-tumor drug carriers (Yan et al. 2019), there is still no detailed comparative analysis of their effects on tumor and normal cells. An important result of this work is the demonstration of significantly greater sensitivity of normal cells, at least at the genome level, to the action of SWCNTs. And this should be taken into account when using carbon nanomaterials for drug delivery to the tumor.

The observed dose-dependent changes in the expression of the studied genes in the normal human astrocytes and glioblastoma cells treated by the SWCNTs indicate for their possible genotoxic effect

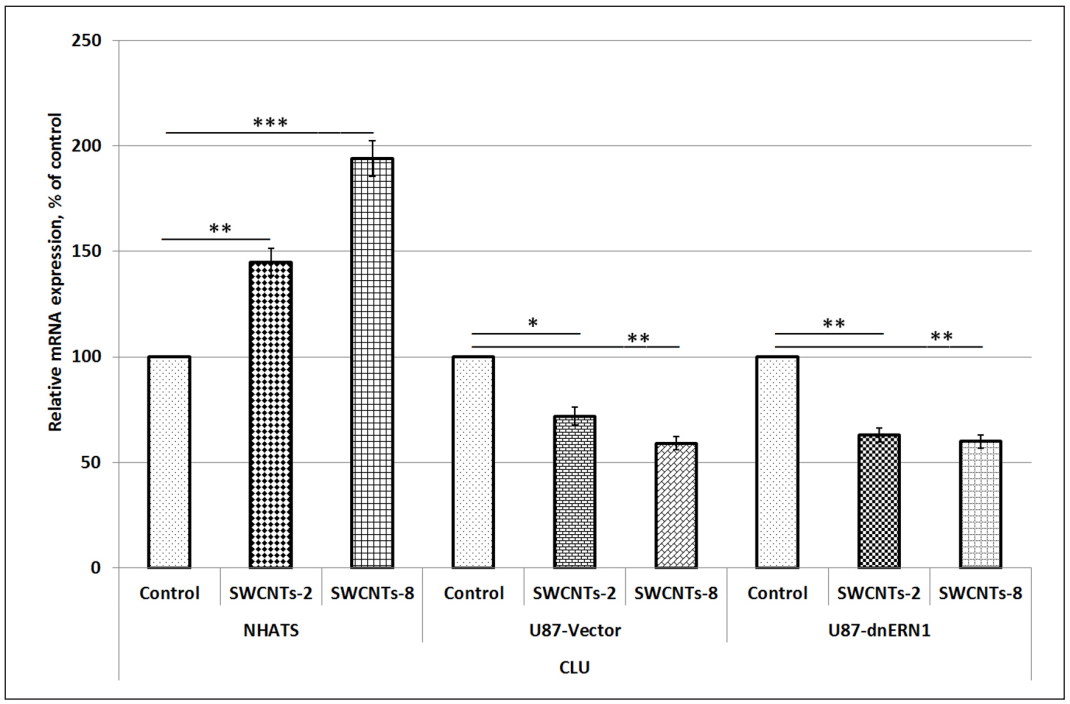


Figure 7. The impact of SWCNTs (2 and 8 ng/ml, for 24 h) on the clusterin (CLU) mRNA expression in normal human astrocytes line NHA/TS and U87 glioblastoma cells stable transfected by empty vector (U87-Vector) or dnERN1 for ERN1 knockdown (U87-dnERN1) versus not treated (control) cells. Values of this mRNA expression were normalized to the ACTB mRNA level; n=4.

that is in agreement with the data toxicity of carbon-based nanomaterials (Toyokuni 2013; Chen et al. 2014, 2020; Francis and Devasena 2018; Kobayashi et al. 2017; Minchenko et al. 2018; Jia et al. 2019; Li et al. 2021; Rudnytska et al. 2021). Furthermore, it is possible that the toxic effect of SWCNTs might be realized through the ER stress, which is consistent with the numerous data concerning the molecular mechanisms of various nanoparticles' impact on the genome function (Chen et al. 2014; Cao et al. 2017; Simon et al. 2017; Hu et al. 2018). Since the functional activity of all studied genes is altered in most malignant tumors and cancer cells, there is a great probability that it may be associated with the ER stress (Jhanwar-Uniyal et al. 2003; Sierko et al. 2007; Amin-Wetzel et al. 2017; Liu et al. 2018; Cao et al. 2020; Tahir et al. 2020; Zhao et al. 2020, 2021; Kim et al. 2021; Minchenko et al. 2021; Patarat et al. 2021; Piras et al. 2022). Furthermore, the genotoxicity and activation of ER stress by carbon nanotubes explains the possible molecular mechanisms responsible for their carcinogenicity (Toyokuni et al. 2013; Minchenko et al. 2021). It is important to note that inhibition of ERN1 signaling deprograms the tumor genome, suppresses cell proliferation and tumor growth *in vivo* in mouse brain after intracranial implantation of U87 glioblastoma cells, expressing the ERN1 dominant negative construct as well as significantly modifying the tumor phenotype and the sensitivity of tumor cells to hypoxia and anti-cancer drugs (Auf et al. 2010; Minchenko et al. 2015, 2021; Avril et al. 2017; Logue et al. 2018).

Most of the proteins encoded by the above-mentioned genes are known as multifunctional proteins that are involved in various cell functions. In this regard, it is difficult to determine the functional significance of those gene expression changes that we found in the normal astrocytes as well as glioblastoma cells treated by the SWCNTs, but we assume that very low quantity of these nanoparticles may dysregulate the genome function through ER stress dependent and other mechanisms. Similar results have also been reported with carbon nanoparticles previously (Minchenko et al. 2018; Rudnytska et al. 2021, 2022).

In conclusion, we revealed that SWCNTs in very small quantities exert a dose-dependent genotoxicity in normal human astrocytes and glioblastoma cells and disturb their genome function through the up-regulation of *DNAJB9* and some other genes encoding the key regulatory proteins. It is possible that SWCNTs affected the expression of studied genes due to ER stress in particular due ERN1 signaling pathway both in normal and tumor cells, but the stress that is constantly present in tumor cells and is responsible for generation of multiple resistances also created partial resistance to the SWCNTs action (Minchenko et al. 2021). Moreover, based on the studied genes, the inhibition of ERN1 signaling pathway in glioblastoma cells almost completely removed the impact of SWCNTs on the expression of most studied genes. It is possible that ER stress and especially ERN1 signaling pathway mediates the SWCNTs action both in normal and tumor cells as well as creation of partial resistance to these nanotubes in glioblastoma cells. Better understanding of the detailed molecular mechanisms of the action of SWCNTs, unique carbon nanomaterial, on both tumor and normal cells is very important not only from the view of its promising therapeutic application, but also its potential risk associated with use for human health. The present data indicate that, just as in other types of nanoparticles, more caution is also needed in the SWCNTs biomedical applications.

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Conflict of interest: *The authors declare no conflict of interest.*

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